THE AMERICAN JOURNAL OF PATHOLOGY

VOLUME XIII SEPTEMBER, 1937 NUMBER 5

ARTERIOLAR SCLEROSIS IN HYPERTENSIVE AND NON-HYPERTENSIVE INDIVIDUALS *

ALAN R. MORITZ, M.D., AND M. R. OLDT, M.D.

(From the Institute of Pathology, Western Reserve University and The University Hospitals, Cleveland, Ohio)

INTRODUCTION

The association of cardiac enlargement and renal contraction has been recognized since Bright's classical case reports in 1836. Shortly thereafter Johnson added diffuse disease of the smallest arteries to the pathological anatomy of Bright's disease and in 1873 proposed the theory that renal disease was primary with subsequent diffuse thickening of the walls of the smallest arteries leading to increased peripheral resistance, elevated blood pressure and cardiac hypertrophy.

Subsequent observations added complexity rather than simplification to the problem. Gull and Sutton reported widespread small vessel disease called by them "arterio-capillary fibrosis," and observed that "these changes are, or may be, independent of renal disease, and that the renal change in chronic Bright's disease with contracted kidneys, when present, is but a part of a general morbid condition." They concluded that the diffuse vascular disease was a primary pathological change responsible for

* Aided by a grant from the Josiah Macy, Jr. Foundation.
Received for publication June 4, 1937.

increased resistance to blood flow. Confirming this general hypothesis were the early clinical observations on blood pressure by Mahomed to the effect that high blood pressure precedes the clinical signs of renal damage.

There were then three divergent views as to the pathogenesis of chronic hypertension: (1) that the renal disease was primary; (2) that the renal disease was but a part of a diffuse primary vascular disease; and (3) that the hypertension itself was primary, the renal and the vascular changes being secondary to it.

The recognition by Jores of two types of contracted kidneys, inflammatory and arteriosclerotic, provided the anatomical basis for differentiating nephritic from essential hypertension. The latter, according to Fishberg's definition which is representative of the majority opinion, is chronic hypertension which neither on clinical nor anatomical grounds can have been caused by preceding inflammatory or obstructive renal disease. It has become generally accepted that the primary change in nephritic hypertension is the renal injury, but the relation of the contracted kidney of nephrosclerosis to hypertension has remained a controversial subject. It is generally believed that the renal vascular disease leads to renal atrophy and contraction but there is a divergence of opinion about the relation as to the cause and effect between the hypertension and the renal vascular disease. Certainly, essential hypertension commonly precedes any clinical evidence of renal insufficiency. Whether or not chronic hypertension precedes the vascular disease that so frequently leads to renal atrophy is the controversial point.

This divergence of opinion as to the relation between essential hypertension and vascular disease may be illustrated by citing the opinions of Volhard and Fahr. Volhard recognized two principal types of hypertension, "pale" and "red." "Pale" hypertension is secondary to renal insufficiency. The renal insufficiency may be due to primary renal disease (glomerulonephritis, and so on) or to the renal vascular damage caused by "red" hypertension, and the "pale" hypertension is the immediate result of the action of pressor substances causing spasm of arterioles throughout the body. "Red" hypertension is primarily a functional derangement to which certain persons are constitutionally predisposed and in which the general distensibility of small arteries is impaired and

blood pressure elevated entirely without primary organic arteriolar contraction. In regard to the "red" or "essential" hypertension his views are based on "the assumption of an early senescence of the whole arterial system, a fatigue of its different structural units, microscopically visible in diminution of internal muscular layer, defects in the elastic layer and over-stretching, all processes commencing while a normal pressure still exists and followed by an increased tonus of the arterioles via vaso-vascularis reflex." This places the cause of essential hypertension outside the kidney except in those instances where the hypertension itself causes renal damage and thus establishes a vicious circle. Fahr, on the other hand, was of the opinion that hypertension is a secondary phenomenon in relation both to inflammatory and to arteriosclerotic renal disease. The primary change is renal and the mechanism by which blood pressure is elevated is, according to him, "compensatory." Bell and Clawson have attempted to harmonize these contradictory views by admitting the absence of any real evidence as to which is antecedent, hypertension or renal vascular disease, but opined that "it is, however, probable that when severe arteriolar sclerosis of the kidney has developed, the circulatory obstruction tends to raise the blood pressure to higher levels."

Critical commentators, such as Hewlett and Boyd, have expressed the belief that the primary change in essential hypertension is functional in the form of arteriolar spasm with resulting increase in peripheral resistance and a later development of permanent structural changes in the form of arteriolar sclerosis which fixes the hypertension by organic narrowing of vessels. Christian and O'Hare felt that the present evidence as to the cause and effect relation of chronic hypertension and arteriolar sclerosis is inadequate to support any definite conclusion as to sequence.

Excluding such possible causes of hypertension as nephritis, urinary obstruction, obesity, hyperthyroidism, pituitary tumor, lead poisoning, adrenal tumor, aortic insufficiency, coarctation of the aorta and arteriovenous aneurysm, there remain the following general conceptions as to the cause and effect relation of arteriolar sclerosis and chronic hypertension:

1. That chronic hypertension is the result of primary renal arteriolar sclerosis, either because a generalized reflex spasm of peripheral vessels is initiated in the ischemic kidneys, or because

of the retention or elaboration of pressor substances incident to a reduced blood flow through the kidney.

2. That chronic hypertension is the result of increased resistance to blood flow caused by widespread primary arteriolar sclerosis with resulting narrowing of vessel lumens.

3. That chronic hypertension is due primarily to arteriolar spasm and that the arteriolar degenerative changes in the kidney and elsewhere are secondary to the increased intravascular pressure and may or may not serve to increase the severity of the hypertension because of organic lumen narrowing.

There is an almost equal lack of agreement as to the character and distribution of vascular lesions in association with essential hypertension. Johnson and Ewald described medial hypertrophy as being a predominant arteriolar change and observed it as occurring generally throughout the body. Gull and Sutton described an equally wide distribution of the arteriolar lesions but believed them to represent degeneration rather than hypertrophy. Jores reported widespread arteriolar intimal hyalinization but failed to find vascular changes in the skeletal muscles. Evans observed medial hypertrophy as well as intimal proliferation and hyalinization in many organs of hypertensive individuals but together with Fishberg and Bell and Clawson failed to discover arteriolar disease in the skeletal muscle. The interest that has been manifested in the occurrence or non-occurrence of arteriolar disease in the skeletal muscle in essential hypertension has been stimulated by the fact that the skeletal muscles constitute so large a part of the peripheral vascular bed. Kernohan, Anderson and Keith reported that the arterioles in skeletal muscles of persons with essential hypertension very frequently showed hypertrophy of the media, proliferation of the intima and reduction of lumen caliber. Scott, Seecof and Hill reported a high incidence of arteriolar lesions in the skeletal muscle of hypertensive patients and Andrus observed more severe fibrosis of the media of arterioles from the skeletal muscles of hypertensive than of non-hypertensive individuals but did not believe that there was wall thickening or reduction of lumen caliber.

The determination of the relation that arteriolar disease bears to chronic hypertension is obviously of more than academic interest especially with the advent of the surgical treatment of the disease.

If permanent organic changes are primary, less can be expected of operations designed to relieve vascular spasm than if the vascular spasm were primary and the organic changes secondary. In the elucidation of the problem it was felt that a clear understanding of the pathological anatomy of hypertension would be of value. Many careful and comprehensive investigations of the pathological anatomy of hypertension have been made and it appeared that the only hope for further morphological or statistical investigations would be the employment of different methods. So far as could be determined no purely objective and yet comprehensive study of the pathological histology, distribution and relative severity of arteriolar lesions in the various organs and tissues of a large number of individuals has been heretofore reported. The correlation of these objective findings with the clinical and anatomical data assembled later was undertaken in the present investigation.

Prior to this objective study of arteriolar disease a general survey was made of the clinical and pathological characteristics of cases of essential hypertension based on the records of the departments of medicine and pathology of Western Reserve University and The University Hospitals.

General Characteristics of the Population of a Representative Chronic Hypertensive Group

The clinical and autopsy records of 200 consecutive cases of chronic hypertension were studied to determine the age, race and sex characteristics of the disease. These were cases in which there was no significant degree of inflammatory heart disease. They were known to have had prolonged elevation of systolic blood pressure over 150 and diastolic pressure over 100 mm. of mercury, and to have had heart weights in excess of 400 gm. in males and 350 gm. in females. In the presence of obliterative coronary arterial disease heart weights in excess of 500 gm. were required for acceptance. No case was included in this series unless death was due to uremia, cardiac decompensation or cerebral hemorrhage.

The group was comprised of:

116	males	with	a	mean	age	of	52	years
84	females	"	"	"	"	"	50	"
60	blacks	"	"	"	"	"	47	" P.E.m. 0.9
140	whites	"	"	"	"	"	55	" P.E.m. 0.5

In this series the mean age of females was 2 years lower than the age of males at the time of death. This was not a statistically significant difference. The mean age of blacks was 8 years lower than that of the whites at the time of death which was 7.5 times greater than the P.E. of the difference.

A comparison of the race and sex population of the hypertensive group to the race and sex population of 1177 consecutive autopsies of all kinds performed on individuals over 30 years of age between the years 1930 to 1935 in the same laboratory is shown below.

	Whites	Blacks	Males	Females
	%	%	%	%
Cases of chronic hypertension	70	30	58	42
General autopsy series	80	20	60	40

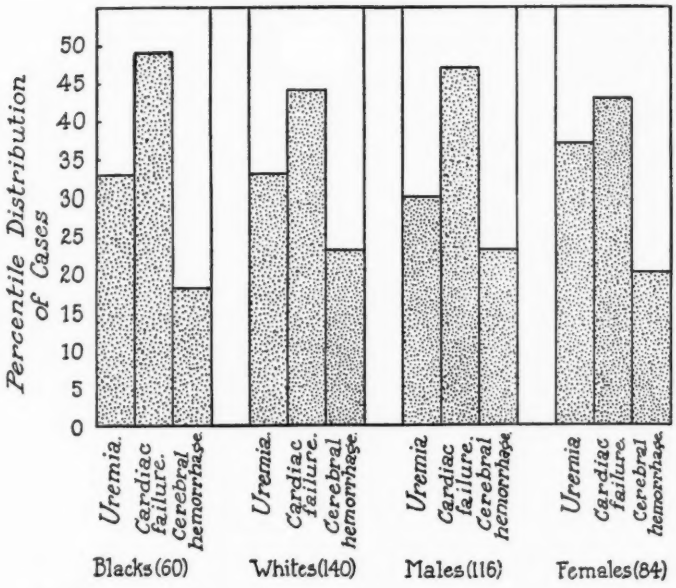
The only significant difference in the population of the chronic hypertensive group as compared to that of the general autopsy series is a racial one. Blacks were encountered with greater relative frequency in the hypertensive group than would be expected from the racial constitution of the general autopsy series. There are 50 per cent more blacks in the hypertensive group than the incidence of the blacks in the general autopsy population would indicate. In contrast there are 13 per cent fewer whites in the hypertensive group than would be expected if whites and blacks had the same degree of mortality from conditions related to chronic hypertension.

Two conclusions appear to be justified as far as this group of 200 cases is concerned: one is that the blacks with chronic hypertension died at a younger age than did whites; and the other is that the percentage of blacks in the hypertensive group was higher than the percentage of whites if the racial constitution of the entire autopsy population was considered.

An investigation was next made of the relation of race and sex to the mode of death in cases of essential hypertension. In the entire group it was found that there were 68 cases in which death was due to uremia, 89 to cardiac decompensation and 43 to cerebral hemorrhage. In Text-Fig. 1 it may be seen that in the different race and sex groups there were no very striking differences in the proportion of each group dead of uremia, cardiac decompensation and cerebral hemorrhage.

The greater susceptibility of blacks over whites to death from

hypertension or its complications was not reflected in the manner in which blacks with hypertension died. About the same percentile proportions of death from uremia, cardiac decompensation and cerebral hemorrhage were seen in blacks, whites, males and females.

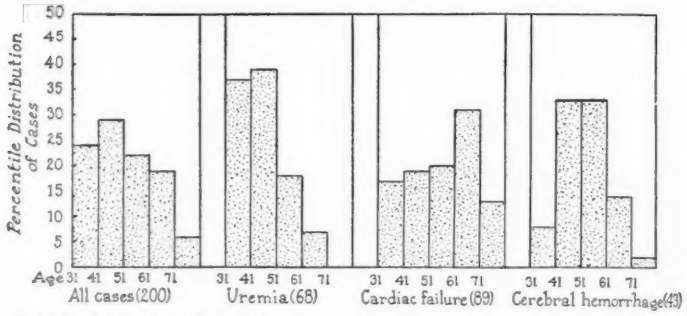


Text Fig. 1.- Relation of race and sex to mode of death in 200 consecutive cases of chronic hypertension.

The correlation of age and mode of death showed striking differences in the age at death of the various types of chronic hypertension. In Text-Fig. 2 it may be seen that the highest incidence of death from uremia was in the 4th and 5th decades. Seventy-five per cent of all the individuals dead from uremia died under 51 years of age. The age incidence was quite different in the groups dead of heart failure and cerebral hemorrhage. In these, death occurred after the age of 51 in 64 per cent and 49 per cent respectively.

Of some significance is the high incidence of obliterative coro-

nary arterial disease in the group of chronic hypertensives dead of heart failure. Forty per cent of all those dead of heart failure were found to have either remote coronary thrombosis or obliterative coronary sclerosis. The incidence of severe coronary arterial disease in the chronic hypertensives dead of cardiac decompensation began at 27 per cent in the 4th decade and rose to 60 per cent in the 7th.



Text Fig. 2. Age at death of 200 consecutive cases of chronic hypertension in which death was due to uremia, cardiac failure or cerebral hemorrhage.

Arteriolar Disease in Hypertensive and Non-Hypertensive Individuals

Selection of Cases: The material on which the major part of this study was made was comprised of 200 cases: 100 had no history of hypertension and the other 100 were known to have had chronic hypertension. Obviously, the possibility of hypertension having been present at some time in some members of the control group could not be positively excluded. The acceptance of a case as non-hypertensive depended on the fulfillment of two criteria. One was that there must have been repeated blood pressure determinations, none of which exceeded 140/90 and the heart weights in males were required to be less than 400 and the females less than 300 gm. As a further means of excluding cardiac hypertrophy, all hearts were examined microscopically and any showing histological evidence of hypertrophy were excluded. All cases selected for the chronic hypertensive group had repeated blood pressure determinations higher than 160/90 or 150/100. No male

was included in this group if the heart weighed less than 450 gm. and no female if the heart weighed less than 350 gm. Cases of inflammatory heart disease were excluded and if severe coronary arteriosclerosis were present, a heart weight in excess of 500 gm. was required for acceptance. The frequent occurrence of cardiac hypertrophy leading to heart weights up to 500 gm. in non-hypertensive individuals as the result of focal myocardial ischemia and dilatation in severe coronary disease has been described by Moritz and Beck. These cases were selected on the basis of the above outlined criteria and with no knowledge as to the presence or absence of renal or vascular disease. The selection was made in such a manner that the population of the two groups was similar in regard to age, sex and color. Approximately one-third of each group was comprised of individuals between 31 and 45 years of age, one-third between 46 and 60, and one-third 61 years of age or over.

Size of Vessels Studied: No satisfactory definition of an arteriole was found, other than that the smallest arteries are arterioles. A variety of criteria have been proposed to distinguish between small arteries and arterioles (Maximow, Cowdry, Benninghoff). These include the number of layers of smooth muscle in the media, the existence of an internal elastic lamella and the external cross sectional diameter of the vessel. We have not found any of these distinctions useful and propose to use the terms "arteriole" and "small artery" interchangeably. This investigation, however, was directed at arteries that had an external diameter of 100 μ or less.

Methods of Investigation: Various staining methods were found useful in a study of the exact anatomical character of certain arteriolar lesions. In addition to hematoxylin-eosin, the most important methods used were Weigert's for elastic tissue and van Gieson's for connective tissue. In studying a particular type of lesion, sections were cut in uninterrupted series and were stained in rotation with hematoxylin-eosin, Weigert's, van Gieson's, the Wilder method for reticulum, and the Mallory-Heidenhain azan-carmine method for connective tissue. In vessels chosen to illustrate certain types of vascular change, the hematoxylin-eosin stained preparation of the vessel was photographed and then the artist made a colored drawing using the photograph for structural

orientation and the slide for color detail. The same section was then decolorized and restained by the van Gieson method. The original drawing served for structural orientation and a second drawing was made again using the slide for color detail. The section was decolorized and restained for elastic fibrils and this time the drawing of the elastic fibrils was either superimposed on the drawing made from the van Gieson preparation or a third drawing of the same vessel was made showing the distribution of the elastic tissue with a neutral counterstain of the rest of the vessel.

Another useful method for the investigation of the pathological anatomy of arteriolar lesions was by means of uninterrupted series of sections. Nine uninterrupted series varying between 100 and 400 in each were prepared from various tissues to study the longitudinal extent and distribution of different types of arteriolar lesions.

Recording of Data: All of the sections of a given organ or tissue in each of the 200 cases were examined objectively with no knowledge as to the presence or absence of hypertension and a record was made as to the kind and severity of the arteriolar disease present. The severity of the arteriolar changes was recorded as mild, moderate or severe by the symbols +, ++ and +++. If the arteriolar disease was focal in a given tissue, a note was made as to the kind of lesion and the fact that it was focal, but such disease was ignored in the general classification of the severity of arteriolar sclerosis in a given tissue. Every organ finally classified as being the seat of mild, moderate or severe arteriolar sclerosis was characterized by the presence of diffuse vascular disease. In many cases of mild, and in some instances of moderate or severe sclerosis, all of the arterioles were not affected, but invariably enough were changed to constitute a general rather than a local process within that organ or tissue.

These degrees of change were not comparable to one another in different tissues. Severe arteriolar sclerosis in a skeletal muscle was not of the same grade of severity or even the same kind of vascular disease as was present in severe arteriolar sclerosis in the spleen. Mild, moderate or severe, refer to the three grades of severity in the particular tissue under consideration.

PATHOLOGICAL HISTOLOGY OF CHRONIC ARTERIOLOSCLEROSIS

Intimal Hyalinization: This was the most common form of chronic arteriolar disease observed and consisted of a subendothelial accumulation of homogeneous, acidophilic material which appeared to represent an infiltration or expansion of the ground substance between smooth muscle of the media and the endothelium. Vacuolar degeneration, either fatty or hydropic, was common and although there was considerable variation in staining reaction of the hyalin, it usually stained deep blue with the Mallory-Heidenhain azan-carmine and the Mallory connective tissue methods, yellow with the van Gieson, and green with the Masson trichrome light green stain. The disposition of hyalin varied, being sometimes in the form of circumscribed subendothelial plaques (Fig. 1), sometimes a subendothelial collar of uniform thickness, and sometimes annular but of irregular thickness, so as to displace the lumen to an eccentric position (Fig. 2). The longitudinal distribution of the hyalin along a vessel was also variable with normal segments interspersed between diseased segments. Not all arterioles with intimal hyalinization had narrowed lumens and some actually appeared to be dilated (Fig. 3). This apparent dilatation was thought to be due to an increased resistance to postmortem contraction imparted to the vessels because of the hyalin, this view being supported by the frequent absence of undulation of the internal elastic lamella of such vessels.

The relation of the internal elastic lamella to the hyalin varied considerably. Although the principal mass accretion of hyalin was commonly inside of the internal elastic lamella (Figs. 1 and 3), it appeared that the hyalin actually enveloped the elastic lamella which in many vessels lay approximately in the center of the hyaline mass (Fig. 2). In such circumstances, elastic degeneration was invariable and was represented by swelling, disruption and dispersion of fibers with eventual complete disappearance.

Medial Hypertrophy and Degeneration: Two types of chronic medial change in the arterioles were observed. One was manifested by increased medial thickness due to an increase in the number or size of smooth muscle cells (Fig. 5), and the other was represented by a relative increase in the amount of intercellular collagen throughout the media (Fig. 6). Although these changes

did occur independently of one another, yet they occurred together (Fig. 6) with such regularity that in the grading of arteriolar lesions according to severity the two changes were treated as though they were part of the same process.

Variations in the degree of postmortem contraction of vessels seen in different individuals or in different vessels of the same individual frequently made it difficult to identify medial hypertrophy. When true hypertrophy of the media had occurred (Fig. 5) the increased thickness of the media was not associated with the same degree of undulation of the internal elastic lamella as was present in normal vessels (Fig. 4), which appeared to have thick walls because of excessive postmortem contraction.

In normal arterioles the media was seen to have three components — smooth muscle, intercellular collagen and occasional irregularly disposed elastic fibers. With the van Gieson method the collagen stained very faintly or not at all in normal arterioles (Fig. 4), but in arterioles the seat of medial degeneration, the increase in collagen was readily recognized (Fig. 6). The increase in collagen was not associated with penetration of the media by fibrocytes, was more pronounced in the inner than in the outer half of the media, and was in some instances associated with atrophy rather than hypertrophy of smooth muscle cells. Medial degeneration showed marked segmental variation, as was the case also in intimal hyalinization. In some segments the medial degeneration was associated with severe secondary degenerative changes so as to cause stenosis of the lumen (Figs. 7 and 8). As a rule this change occurred independently of any intimal disease.

Endothelial Hyperplasia: Endothelial hyperplasia regularly resulting in reduction in lumen caliber was seen most frequently in vessels over 50 μ in diameter, occasionally in arterioles down to 30 μ in diameter, but rarely under this. As a rule the hyperplastic endothelium of the larger arterioles gave way gradually to intimal hyalinization in the smaller arterioles. It was not possible to recognize endothelial proliferation with certainty when all of the endothelium was represented by lining cells. In such circumstances postmortem contraction of the vessels together with tangential section could give an illusion of endothelial hyperplasia. The piling up of endothelial cells layer on layer did, however, present a clear picture of endothelial hyperplasia (Figs. 9 and 10).

Such hyperplasia was in rare instances superimposed on a relatively unaltered internal elastic lamella (Fig. 9). More commonly, however, there was formation of new elastic fibers between the hyperplastic endothelial cells. In some vessels this new elastic tissue formed an irregular intercellular mesh, which in others was seen to be organized to form more or less concentric lamellas, the lamellas being separated from one another by newly formed endothelial cells (Fig. 10). As in the case of the media, there was no recognizable penetration of the intima by fibrocytes although the proliferated endothelial cells tended to become spindle shaped so as to resemble fibrous connective cells closely. As in the case of intimal hyalinization, the elastic fibers, both new and old, showed a pronounced tendency to degeneration, as manifested by swelling, disruption and dispersion of fibrils. This degeneration of elastic fibers was especially pronounced when the proliferated endothelium underwent hyalinization, which was a common secondary degenerative change in vessels affected in this manner (Fig. 10). A wide variety of acute secondary degenerative changes was seen and will be described later.

Pathological Changes in Arterioles of Non-Hypertensive Individuals

As already indicated, this study was directed at chronic rather than acute arteriolar changes. Acute primary degenerative and inflammatory arterial and arteriolar disease have been discussed recently by Karsner and were not studied in this investigation.

The incidence and relative severity of arteriolar sclerosis in the various organs and tissues in non-hypertensives is summarized in Table I. The table does not include a record of the vascular changes in the female internal genitalia where cyclic hyperplasia and involution make their occurrence exceedingly common, or changes in various tissues not examined with sufficient frequency to make statistical comparisons profitable. The heart and lungs were not included in the table because of the rarity of arteriolar changes in those situations in this series of cases. Pulmonary (Parker and Weiss) and myocardial (Karsner and Bayless) arteriolar sclerosis is seen most commonly in cases of rheumatic carditis and since no individuals with cardiac enlargement were

TABLE I

The Relation of Age, Sex and Race to the Incidence and Severity of Arteriolar Sclerosis in Non-Hypertensive Individuals

Age Groups	Spleen			Pancreas			Adrenal			Gastro-Intestinal			Brain *			Skeletal muscle			Liver			Kidney		
	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total
yrs.	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
31–45	56	17	73	38	0	38	25	0	25	12	0	12	..	..	..	17	0	17	3	0	3	7	0	7
46–60	63	19	82	33	6	39	39	5	44	16	0	16	..	..	..	20	0	20	6	0	6	9	0	9
61 and over	74	26	100	50	5	55	41	12	53	27	0	27	..	..	..	13	0	13	24	5	29	16	0	16
Total	65	21	86	38	5	43	36	6	42	19	0	19	17	0	17	15	0	15	11	2	13	12	0	12
Sex Groups																								
Male	63	23	86	43	6	49	40	7	47	18	0	18	..	..	..	12	0	12	10	2	14	12	0	12
Female	68	17	85	32	2	34	31	4	35	20	0	20	..	..	..	21	0	21	13	2	15	10	0	10
Racial Groups																								
White	65	20	85	37	7	44	33	6	39	21	0	21	..	..	..	13	0	13	10	2	14	13	0	13
Black	66	22	88	40	0	40	40	5	45	15	0	15	..	..	..	18	0	18	17	4	12	10	0	10

* Brain examined in 29 cases only.

included in the control group most cases of rheumatic heart disease were automatically excluded.

Distribution of Arteriolar Sclerosis: An examination of the total incidence of arteriolar sclerosis in the various tissues of non-hypertensives showed a high incidence in the spleen (86 per cent), pancreas (43 per cent) and adrenal capsules (42 per cent). Of all the tissues recorded in Table I, the renal arterioles were found to be least frequently diseased. There were 12 instances of renal arteriolar sclerosis in the entire control group, and of these, the sclerosis was classified as mild in 10 and moderately severe in 2.

Occurrence of Various Histological Types of Chronic Arteriolar Changes in Non-Hypertensive Individuals

Intimal Hyalinization: All three anatomical types of chronic arteriolar disease were seen. The most common was intimal hyalinization. In the control group this was more pronounced in vessels over 50 μ in diameter. It was found most commonly in the spleen, pancreas, the capsules of the adrenals, brain, eye and kidney. It occurred in vessels of the internal genitalia and in the hyperplastic intima of arterioles in regions the seat of chronic or healed inflammation. It was rarely found in the gastro-intestinal tract except in association with inflammation, in liver, skeletal muscle, subcutaneous tissue, lymph nodes or bone marrow.

Medial Hypertrophy and Degeneration: Medial hypertrophy and degeneration were seen in the gastro-intestinal tract in 19 per cent and in the skeletal muscle of 15 per cent of the non-hypertensives. In no instance was it graded as a severe change. This type of arteriolar disease was seen frequently in the spleen but was usually obscured by the more striking intimal disease.

Endothelial Hyperplasia: Intimal endothelial hyperplasia in the arterioles of the non-hypertensive group was seen principally in association with involutional or inflammatory changes. It was seen almost constantly in the ovary, uterus and Fallopian tubes. It was seen in the adventitial arterioles in syphilitic aortitis and in association with chronic peptic ulcer, cholecystitis and appendicitis and a variety of other chronic inflammatory conditions. Medial degeneration in the form of intercellular collagen as well as intimal hyalinization were frequently associated changes.

* Brain examined in 29 cases only.

Age and Sex Differences: Table I shows the relation of age to the incidence and severity of arteriolar sclerosis in the non-hypertensive group. In practically all of the tissues examined the arteriolar lesions were seen with greater frequency and severity as age advanced. There were no consistent differences between males and females or between blacks and whites in regard to incidence or severity of arteriolar sclerosis.

Pathological Changes in Arterioles of Hypertensive Individuals

The incidence and severity of arteriolar sclerosis in the various organs and tissues of the hypertensive group are shown in Table II. As in the case of Table I, the internal genitalia are not included because of the common occurrence of vascular changes associated with cyclic involution and hyperplasia. The heart and lungs were omitted because of the rarity of chronic arteriolar changes in these situations in hypertensives except in association with rheumatic heart disease.

Distribution and Severity of Arteriolar Lesions: An examination of the total incidence of arteriolar sclerosis in the various tissues shows approximately the same frequency in the spleen and the kidney, 98 per cent and 97 per cent respectively. In the hypertensive group there was approximately the same sequence of organs, according to the incidence of arteriolar disease, except for the kidney, which changed from being one of the organs least frequently the seat of arteriolar sclerosis in the non-hypertensives to sharing first place with the spleen in being most frequently the seat of arteriolar sclerosis. In every organ and tissue, however, there was an increase in both frequency and severity of arteriolar disease throughout the hypertensive group as compared to the controls.

In grading, arteriolar sclerosis was designated as mild, moderate or severe. In the hypertensive group, severe arteriolar sclerosis was observed in the kidneys (47 per cent), gastro-intestinal tract (15 per cent), skeletal muscle (14 per cent), liver (11 per cent) and brain (8 per cent), in contrast with the non-occurrence of severe arteriolar sclerosis in any of those situations in members of the control group. Although severe vascular lesions were ob-

TABLE II

The Relation of Age, Sex and Race to the Incidence and Severity of Arteriolar Sclerosis in Hypertensive Individuals

Age Groups	Spleen			Pancreas			Adrenal			Gastro-Intestinal			Brain *			Skeletal muscle			Liver			Kidney		
	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total	Mild or moderate	Severe	Total
yrs.	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%	%
31–45	46	54	100	21	52	73	51	39	90	44	20	64	..	..	..	64	17	81	38	9	47	33	67	100
46–60	39	61	100	28	62	90	56	38	94	64	15	79	..	..	..	62	25	87	36	19	55	46	50	96
61 and over	44	52	96	56	31	87	50	44	94	48	7	55	..	..	..	48	3	51	41	6	47	72	22	94
Total	44	55	99	40	47	87	51	42	93	55	15	70	42	8	50	58	14	72	38	11	49	50	47	97
Sex Groups																								
Male	50	50	100	42	42	84	51	37	88	60	10	70	..	..	..	58	12	70	36	7	43	47	51	98
Female	37	60	97	38	53	91	51	49	100	50	20	70	..	..	..	62	16	78	40	18	58	54	41	95
Racial Groups																								
White	45	55	100	38	48	86	52	42	94	55	19	74	..	..	..	55	18	73	33	15	48	45	51	96
Black	42	54	96	46	43	89	48	42	90	55	7	62	..	..	..	68	4	72	48	3	51	59	41	100

* Brain examined in 33 cases only.

served in the spleen, pancreas and capsules of adrenals of some of the controls, the incidence of severe arteriolar sclerosis was more than doubled in those same organs in the hypertensive group.

Age and Sex Differences: In contrast with the control group in which the occurrence and severity of vascular disease increased with age, the general trend in the hypertensive group was for vascular disease to be less severe in the older individuals. An especially striking drop in the severity of arteriolar sclerosis with age was seen in the kidney, where the vascular disease was severe in 67 per cent of all hypertensives dead under the age of 45, in 50 per cent of hypertensives dead between the ages of 46 and 60, and in only 22 per cent of hypertensives dead after the age of 61 years.

No significant differences in the occurrence or severity of arteriolar disease could be seen in males and females or in blacks and whites within the hypertensive group.

Occurrence of Various Histological Types of Arteriolar Sclerosis in Hypertensive Individuals

Endothelial Hyperplasia: In a discussion of the anatomical types of arteriolar disease seen in the various tissues in the hypertensive group the occurrence of intimal proliferation not demonstrably caused by inflammation of surrounding tissues appeared to deserve first consideration. This type of change was most pronounced in renal vessels over 30 μ in diameter, giving way to intimal hyalinization in the afferent arterioles. It appeared frequently to be associated with medial degeneration (intercellular collagen and thinning), and in many instances was complicated by hyaline, fat, mucoid or chromatropic degeneration, or necrosis of the hyperplastic intima as well as of the media. Although primary intimal hyperplasia was observed most frequently in the kidneys, it was seen occasionally in a variety of other situations. In no instance was it encountered in the skeletal muscle, subcutaneous tissue, lymph nodes or bone marrow, except in association with obvious local inflammation.

Acute Arteriolar Lesions: Acute degenerative, necrosing and inflammatory lesions were frequently superimposed on chronic arteriolar lesions of all kinds and were seen most frequently (38

per cent of all cases of chronic hypertension) in the renal arterioles, especially those showing intimal hyperplasia. The lesions varied greatly as to character and severity and were so obviously secondary that although they may have been significant in relation to the rapidity with which the disease progressed, they were not considered significant in relation to the inception of either the hypertension or the renal vascular disease. No tissue or organ was immune from the occurrence of these vascular lesions and, next to the kidney, they were seen most frequently in the gastro-intestinal tract.

Intimal Hyalinization: Intimal hyalinization occurred in the same tissues in the hypertensive as in the non-hypertensive group but with greater frequency and severity in the former. It was seen commonly as a generalized vascular disease in such tissues as the kidney, gastro-intestinal tract, liver and brain, where its occurrence in any degree of severity in non-hypertensives was rare. In the kidney and eye it was frequently associated with endothelial hyperplasia in larger arterioles (50 to 100 μ in external diameter). There was no qualitative difference in the intimal hyalin in the arterioles of the hypertensive and the non-hypertensive groups, although secondary degenerative changes were more common in the former.

Medial Hypertrophy and Degeneration: Medial hypertrophy and collagenous degeneration were widespread and common types of arteriolar changes in the hypertensive group. Without complicating intimal disease these changes were seen most frequently in the skeletal muscle. In a number of instances in which samples of more than one skeletal muscle from the same individual were examined, there appeared to be no significant difference in the severity of the vascular disease in various portions of the skeletal muscular system, with the possible exception of the diaphragm. Sections were prepared from the pectoralis major, rectus abdominis, quadriceps femoris, psoas and diaphragm from 6 individuals having severe generalized arteriolar sclerosis. With the exception of the diaphragm, the various muscles exhibited, as nearly as could be judged, the same degree of arteriolar sclerosis. In 3 of the 6, the diaphragmatic arterioles either appeared normal or mildly sclerotic, whereas in other muscles moderate or severe arteriolar sclerosis was present.

Measurements of Wall Thickness of Arterioles

The claims of Keith, Barker and Kernohan to the effect that the thickening of the walls of the arterioles of skeletal muscle, due principally to medial hypertrophy, could be demonstrated by measurements in microscopic sections, deserved corroboration if their assumptions were correct. Their claims were made on a basis of measuring several arterioles from each of 50 control cases and from 143 cases of chronic hypertension. They found that arteriolar thickening was frequently present in hypertensive individuals and described five different types of chronic hypertension in which different mean wall to lumen ratios were observed. They did not state what the error of the method was or what the observed anatomical variations were. Bell, as well as Andrus, has denied the probability of medial hypertrophy of arterioles in the skeletal muscle of chronic hypertensives, but neither has presented quantitative data to refute the findings of Keith, Barker and Kernohan.

Variations in Measurements of Arterioles Not Related to Disease

To determine the extent of the variation in arteriolar measurements incident to errors in mensuration or to differences in relative wall thickness related to different degrees of postmortem contraction or to actual anatomical differences, 15 samples of 75 arterioles each from the pectoral muscle of 1 non-hypertensive individual were measured and the mean external diameter, the mean lumen diameter and the mean ratio of wall thickness to lumen diameter were calculated. The 15 samples were fixed and prepared for microscopic examination by a uniform technic. The vessels were measured by the method described by Kernohan, Anderson and Keith, using an 8 mm. objective and a X 7 ocular containing a micrometer scale. Every arteriole between 18 and 72 μ in diameter was measured as it was encountered in cross section until 75 vessels had been measured. The outermost cells of the media were used to define the external diameter. To avoid measuring the same arteriole in succeeding sections, the sections from each block of muscle were cut at intervals of between 0.5 and 1.0 mm.

The means of the arteriolar measurements of the 15 samples of 75 arterioles each are given in Table III. This record indicates that the least variable mean was that of the external diameter of

a sample of arterioles having a fixed size range. In this instance it was 31.9 μ with a probable error of 1.5 μ. The variation of the wall to lumen ratio was great, ranging from 1.0–1.4 to 1.0–2.5. It is apparent then that any difference in the wall to lumen ratio within the limits of 1.0–1.4 and 1.0–2.5 in 2 samples of 75 arterioles each, or less, would have no significance unless some more accurate method of measurement were employed than was used in

TABLE III

Record of Measurements of 15 Samples With 75 Arterioles in Each From the Pectoral Muscle of 1 Non-Hypertensive Individual

Sample	Mean external diameter	P.E.m.	Mean internal diameter	P.E.m.	Wall to lumen ratio
	μ		μ		
1	33.4	1.04	16.0	0.58	1 to 1.8
2	31.4	1.08	17.4	0.76	1 to 2.5
3	32.2	0.90	15.5	0.54	1 to 1.9
4	33.8	0.97	17.9	0.72	1 to 2.3
5	31.4	0.83	15.3	0.36	1 to 1.9
6	34.0	1.30	17.1	0.72	1 to 2.0
7	30.1	1.01	15.2	0.54	1 to 2.0
8	32.5	1.08	16.7	0.72	1 to 2.1
9	28.8	0.97	13.8	0.54	1 to 1.8
10	29.4	0.90	15.0	0.54	1 to 2.1
11	30.2	0.97	15.0	0.76	1 to 2.0
12	31.1	1.01	16.1	0.65	1 to 2.0
13	31.8	0.97	15.8	0.61	1 to 2.0
14	30.7	0.86	17.1	0.58	1 to 2.5
15	30.1	1.04	15.9	0.61	1 to 2.2

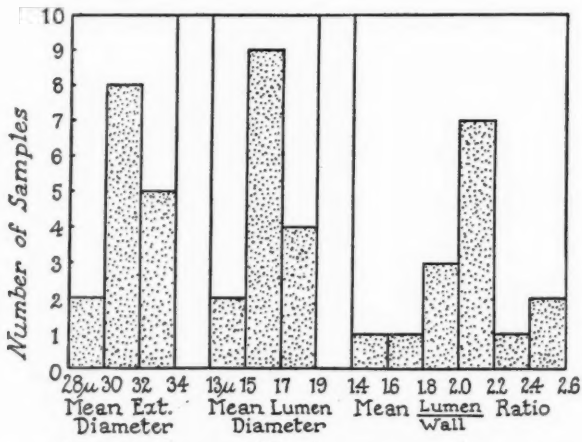
Mean external diameter = 31.4 μ P.E. $\pm$ 1.5
Mean internal diameter = 16.0 μ P.E. $\pm$ 1.0
Mean wall to lumen ratio = 1 to 2.0

this investigation. With such variation, it hardly seemed likely that the differences in the first decimal between mean ratios of wall thickness to lumen diameter in samples of undefined magnitude, such as reported by Keith, Barker and Kernohan, were significant. The distribution of each of the three means for the 15 samples is shown in Text-Fig. 3.

Selection of Control and Hypertensive Cases for Arteriolar Measurements

It was felt from this test of multiple samples from 1 individual that many samples of arterioles from the skeletal muscle of hyper-

tensives and non-hypertensives would have to be measured in order to determine whether real differences in relative wall thickness exist or not. Accordingly, 38 classical cases of essential hypertension, with death from renal insufficiency, heart failure or cerebral hemorrhage, were selected for study. These were about equally divided between males and females, between blacks and



Text Fig. 3. Histograms showing the distribution of 15 samples of pectoral muscle from one non-hypertensive individual according to the mean external diameter, the mean lumen diameter and the mean wall to lumen ratio of arterioles in each sample. Seventy five arterioles ranging between 18 and 72μ in external diameter constitute a sample.

whites, and between persons over and under 45 years of age. A control group of non-hypertensive individuals with comparable age, sex, and race population was selected. As in the case of previously reported measurements, the external and internal diameter of each of 75 arterioles within the limits of 18 and 72 μ in external diameter in samples of pectoral muscle from each of the 76 individuals were measured. These data are shown in Table IV.

TABLE IV

Record of Measurements of 75 Arterioles From the Pectoral Muscle of Each of 38 Non-Hypertensive and 38 Hypertensive Individuals

Non-hypertensive individuals						Hypertensive individuals						
Age	Sex	Color	E.D.	I.D.	Ratio	Age	Sex	Color	Cause of death	E.D.	I.D.	Ratio
yrs.			*μ*	*μ*		*yrs.*				*μ*	*μ*	
43	F	W	31.7	15.1	1 to 1.7	37	M	B	R	38.2	11.5	1 to 0.9
32	F	B	29.2	11.9	1 to 1.4	59	M	W	H	41.8	15.8	1 to 1.2
41	M	W	33.8	18.0	1 to 2.3	50	F	B	H	33.8	14.8	1 to 1.5
46	F	W	32.4	16.2	1 to 2.0	43	F	B	R	37.8	16.2	1 to 1.5
48	F	W	30.6	11.9	1 to 1.2	50	M	B	H	36.7	14.4	1 to 1.3
53	M	W	34.2	19.1	1 to 2.5	54	M	B	C	44.3	19.1	1 to 1.6
38	M	B	34.9	19.8	1 to 2.5	38	F	W	C	46.1	14.0	1 to 0.9
36	F	W	34.2	16.6	1 to 1.9	50	M	B	C	39.2	10.4	1 to 1.1
36	F	W	36.4	21.6	1 to 2.9	38	F	W	H	31.3	13.7	1 to 1.6
48	F	W	30.2	15.1	1 to 2.0	49	F	B	H	33.5	14.4	1 to 1.5
38	M	W	31.3	15.5	1 to 2.0	58	M	B	R	41.0	16.2	1 to 1.3
50	M	W	34.9	18.7	1 to 2.3	54	F	B	H	34.2	12.6	1 to 1.2
57	M	W	30.2	15.5	1 to 2.1	33	F	B	R	34.6	14.8	1 to 1.5
52	M	W	36.4	18.0	1 to 2.0	40	F	B	H	36.7	17.6	1 to 1.9
40	M	W	31.3	16.6	1 to 2.3	58	M	W	H	39.2	20.2	1 to 2.1

E.D. = Mean external diameter of 75 arterioles varying from 18 to 72 *μ* in external diameter.
I.D. = Mean internal diameter of 75 arterioles.
Ratio = Unweighted ratio of mean wall thickness to mean lumen diameter of 75 arterioles.
Cause of death: H = heart failure.
R = renal failure.
C = cerebral hemorrhage.

TABLE IV — *Continued*

Non-hypertensive individuals						Hypertensive individuals						
Age	Sex	Color	E.D.	I.D.	Ratio	Age	Sex	Color	Cause of death	E.D.	I.D.	Ratio
yrs.			μ	μ		*yrs.*				μ	μ	
57	M	W	32.8	13.0	1 to 1.3	58	M	W	H	34.2	13.3	1 to 1.3
36	F	W	33.1	16.2	1 to 1.9	50	F	B	C	33.8	16.2	1 to 1.8
62	M	W	30.2	11.5	1 to 1.2	41	M	B	H	39.2	13.3	1 to 1.0
49	M	W	30.2	15.8	1 to 2.2	51	F	B	R	38.6	16.9	1 to 1.5
58	F	W	32.8	16.9	1 to 2.1	40	M	W	H	37.1	17.6	1 to 1.8
34	M	B	30.6	15.1	1 to 1.9	40	F	B	C	36.4	18.4	1 to 2.0
41	M	W	36.0	16.2	1 to 1.6	37	F	W	R	41.8	14.8	1 to 1.1
44	M	W	30.2	16.2	1 to 2.3	36	M	B	R	36.4	14.4	1 to 1.3
55	F	W	32.8	13.7	1 to 1.4	34	M	B	H	36.0	16.2	1 to 1.6
49	F	W	32.0	16.9	1 to 2.2	36	M	W	C	32.8	10.8	1 to 1.0
37	F	W	29.2	14.0	1 to 1.8	55	M	W	H	43.6	20.2	1 to 1.7
54	M	W	30.6	14.4	1 to 1.9	50	F	B	R	34.2	15.1	1 to 1.6
58	F	W	36.0	18.7	1 to 2.2	44	M	W	R	41.8	13.3	1 to 0.9
34	M	B	32.4	16.9	1 to 2.2	45	M	W	R	46.1	17.6	1 to 1.2
34	M	B	33.1	16.6	1 to 2.0	37	F	W	R	42.5	15.5	1 to 1.1
45	M	W	33.5	17.6	1 to 2.2	36	F	B	R	42.1	15.5	1 to 1.2
50	M	W	29.9	11.9	1 to 1.3	31	M	W	R	36.4	13.0	1 to 1.1
35	F	W	28.1	13.3	1 to 1.8	48	F	W	R	47.2	14.8	1 to 0.9
38	F	B	32.4	16.6	1 to 2.1	36	F	W	H	36.7	15.1	1 to 1.4
38	F	B	29.5	12.6	1 to 1.5	58	F	B	C	33.8	16.2	1 to 1.8
52	M	W	33.5	17.3	1 to 2.1	51	M	W	H	39.6	20.5	1 to 2.1
59	M	B	29.2	13.0	1 to 1.6	53	M	W	H	35.6	13.3	1 to 1.2
40	M	B	33.8	16.2	1 to 1.8	45	F	W	H	44.6	16.6	1 to 1.2

Comparison of Arteriolar Measurements in Control and Hypertensive Groups

A comparison of the means of the mean external diameters for each group (hypertensives and non-hypertensives) shows:

32.2 P.E. ± 2.2 μ for the controls

and

38.4 P.E. ± 4.1 μ for the hypertensives

The difference is significant.

A comparison of the means of the mean lumen diameters for each group shows:

15.8 P.E. ± 2.3 μ for the controls

and

15.4 P.E. ± 2.4 μ for the hypertensives

The difference is not significant.

A comparison of the means of the mean wall to lumen ratio shows:

1.0–1.9 for the controls

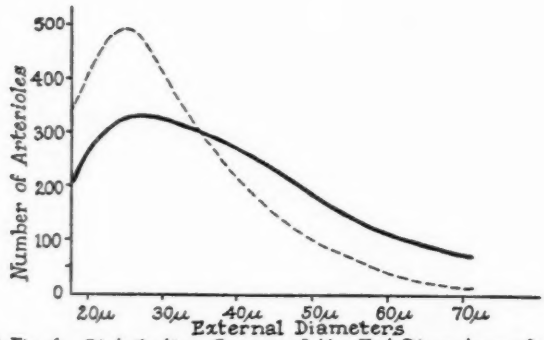
and

1.0–1.36 for the hypertensives.

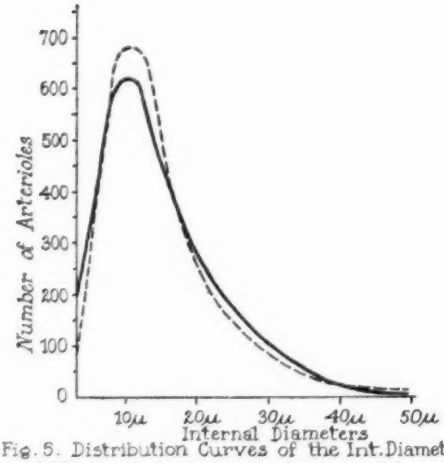
The difference is significant.

So far as the means of the various measurements of the arterioles of the hypertensive and the non-hypertensive groups are concerned, it appeared that the arterioles of the pectoral muscle of hypertensive individuals have relatively thicker walls than those of the non-hypertensives, that the arterioles are larger, as indicated by the mean external diameter, but that there is no real evidence that the arteriolar lumens of the hypertensives have been reduced.

A comparison of the external and internal diameters of arterioles in hypertensive and non-hypertensive individuals is shown in Text-Figs. 4 and 5. In Text-Fig. 4 the external diameters of 2850 arterioles of hypertensives and a like number of arterioles from non-hypertensives have been plotted against the number of vessels in each size group to establish two distribution curves. It may be seen that there are many more arterioles in the small vessel group



Text Fig. 4. Distribution Curves of the Ext. Diameters of:
——2850 arterioles in pectoral muscle of hypertensive individuals.
---2850 " " " " " non- " "



Text Fig. 5. Distribution Curves of the Int. Diameters of:
—— 2850 arterioles in pectoral muscle of hypertensive individuals.
--- 2850 " " " " " non- " "

75 arterioles ranging from 18 to 72μ in external diameter from each of 38 hypertensive and 38 non-hypertensive individuals, were measured.

(18 to 36 μ in external diameter) in the controls than in the hypertensives, and that over an external diameter of 36 μ, vessels are more numerous in the hypertensive groups. This flattening of the curve represents an increase in the mean external diameter of arterioles in the hypertensive group.

In Text-Fig. 5 the internal diameters of the same arterioles have been plotted as abscissae against the number of vessels in each size group as ordinates. There is practically no difference in the two curves, which indicates that in so far as the two groups of cases (hypertensives and non-hypertensives) are concerned, the internal diameters of arterioles in the pectoral muscles are not significantly different.

No deductions can be drawn as to the relative patency of the two groups of arterioles in life. It is possible that medial degeneration rendered the arterioles in the pectoral muscle of hypertensives relatively resistant to postmortem contraction, so that although they appeared to have the same degree of patency as the more fully contracted normal arterioles in tissue sections, they may have had narrower lumens than normal vessels in life. It is equally possible that they were more fully contracted than normal arterioles and that the increased wall thickness in death is a reflection of a relatively greater lumen diameter in life. Differences in the functional state of arterioles in the skeletal muscle of hypertensive and non-hypertensive individuals cannot be conclusively determined from differences in measurements of postmortem material.

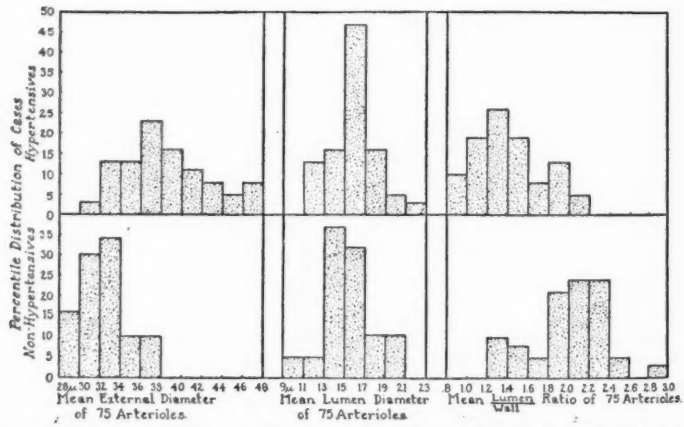
Table V shows the results of the arteriolar measurements in the various subgroups of the 38 hypertensive individuals. It is seen that so far as mean internal and external diameter, and mean wall to lumen ratio are concerned, there are no significant differences between individuals under and over 45 years of age, between males and females, blacks and whites, or between those dead of uremia, heart failure or cerebral hemorrhage.

Finally, in this consideration of the measurements of arterioles, attention was turned to the possible value of measuring arterioles in samples of pectoral muscle to distinguish between hypertensive and non-hypertensive individuals. Text-Fig. 6 was prepared to show the correlation of mean external and lumen diameter values, as well as mean ratios of wall to lumen thickness with chronic hypertension.

TABLE V

Hypertensive Group
Skeletal Muscle

	No. of cases	Age	E.D.	I.D.	Wall to lumen ratio
		yrs.	*μ*	*μ*	
Entire hypertensive group	38	43.8	38.4	15.4	1 to 1.3
31 to 45 years of age	20	38.4	38.7	15.0	1 to 1.3
46 to 60 years of age	18	49.7	37.6	15.8	1 to 1.4
Male	19	43.7	38.2	15.4	1 to 1.4
Female	19	43.9	37.9	15.4	1 to 1.4
White	18	41.7	39.9	15.6	1 to 1.3
Black	20	45.7	37.0	15.2	1 to 1.4
Death from heart failure	17	46.6	37.2	15.5	1 to 1.4
Death from renal insufficiency	14	41.9	39.9	15.0	1 to 1.2
Death from cerebral hemorrhage	7	46.6	38.1	15.0	1 to 1.3


Percentile Distribution of Cases
Hypertensives
Non-Hypertensives
Mean External Diameter of 75 Arterioles.
Mean Lumen Diameter of 75 Arterioles.
Mean Lumen/Wall Ratio of 75 Arterioles.

Text Fig 6. Histograms showing the percentile distribution of 38 control and 38 hypertensive cases, according to the mean external diameter, the mean lumen diameter and the mean wall to lumen ratio of arterioles in samples of pectoral muscle from each. Seventy five arterioles ranging between 18 and 72μ in external diameter constitute a sample.

It was found that in 48 per cent of the hypertensives the mean external diameter of the 75 vessel samples was greater than that found in any of the control group. If the control cases were representative normals it might be said that whenever the mean external diameter of a sample of 75 arterioles, ranging between 18 and 72 μ in external diameter, exceeded 38 μ the sample was from a chronic hypertensive individual. Moreover, in almost half of the group of the chronic hypertensives the mean external diameter was greater than 38 μ. The correlation of mean external diameter and a state of normal blood pressure was less striking. Sixteen per cent of the non-hypertensives had mean external diameters lower than those seen in any hypertensive. So far as these data were representative, it appeared that when the mean external diameter fell below 30 μ chronic hypertension could be excluded.

There was no significant difference between the contour of the distribution curves or the means of the internal diameters of the same 75 vessel samples of pectoral muscle of the hypertensive and non-hypertensive groups.

It has already been stated that the mean wall to lumen ratio of these same samples of arterioles was 1.0–1.9 in the controls and 1.0–1.36 in the hypertensives. The distribution curves of these ratios (Text-Fig. 6) showed a wide overlapping with only 29 per cent of the hypertensive group having a wall to lumen ratio less than that seen in any of the controls. It appeared that if the wall to lumen ratio of a sample of arterioles, such as were measured in this study, was less than 1.0–1.2, that sample was invariably from a hypertensive individual. The distribution curves showed that if the wall to lumen ratio was greater than 1.0–2.2, chronic hypertension could be excluded. Hypertension could be excluded in 32 per cent of the normals by this criterion.

Variations in Arteriolar Measurements Related to Differences in Methods of Tissue Preparation

It was thought that differences in the time at which tissues were fixed in relation to death, differences in the duration of fixation, dehydration and clearing and differences in the time in the paraffin oven might lead to measurable differences in the dimensions of arterioles. To determine whether this were true or not, 8 samples

of pectoral muscle obtained from 1 non-hypertensive individual were prepared for histological examination by the various methods indicated in Table VI. Unless otherwise indicated the tissue was fixed during rigor and kept in 10 per cent formalin for 24 hours. The standard time allowed for dehydration and clearing was 12 hours and for embedding (paraffin oven) 3 hours. All sections were cut at a thickness of 6 μ and stained in the same manner. Seventy-five arterioles ranging from 18 to 72 μ in external diameter were measured in each sample.

Table VI shows that there were no differences between the means greater than the differences observed in samples subjected

TABLE VI

A Comparison of the Mean External Diameter, Mean Internal Diameter, and Mean Wall to Lumen Ratio of Arterioles in 8 Samples (75 Arterioles Between 18 and 72 μ in External Diameter in Each Sample) From the Pectoral Muscle of 1 Non-Hypertensive Individual, to Show the Effect of Variations in Methods of Tissue Preparation On the Dimensions of Arterioles

Sample	Treatment	Mean external diameter	Mean internal diameter	Wall to lumen ratio
		μ	μ	
1	Fixed before onset of rigor	34.9	17.7	1 to 2.1
2	Fixed during rigor	31.6	16.2	1 to 2.1
3	Fixed after dissipation of rigor	32.8	15.8	1 to 1.9
4	Fixed for 168 hours	36.0	20.3	1 to 2.6
5	Dehydration and clearing for 6 hours	31.5	15.1	1 to 1.8
6	Dehydration and clearing for 62 hours	31.5	14.4	1 to 1.7
7	In paraffin oven for 1½ hours	32.3	15.2	1 to 1.8
8	In paraffin oven for 24 hours	34.6	18.3	1 to 2.2

to a uniform method of tissue preparation (see Table III and Text-Fig. 3.) Although this experiment does not prove that variations in technic do not influence the size of vessels in microscopic preparations, yet it does indicate that the anatomical variations and errors in mensuration under controlled conditions account for as great differences as result from deliberate variations in methods of tissue preparation.

Variations in Arteriolar Measurements Related to Differences in the Physiological State of the Vascular System

It was thought that differences in the functional state of arterioles (vasoconstriction or vasodilatation) at the time of death or at the time that the biopsy was taken might be reflected in differences in relative wall thickness in microscopic preparations. To determine whether this were true or not the following experiments were conducted on 4 dogs.

The dogs were mongrels, ranged from 8 to 12 kg. in weight, were mature but were of unknown ages. Each animal was anesthetized with ether and a carotid canula was inserted and connected with a mercury manometer and the systolic blood pressure recorded on a kymograph. As soon as the blood pressure had reached a fairly constant plateau, a biopsy of skeletal muscle was taken. Following this biopsy, the injection of 0.5 cc. per kilo of 1:1000 adrenalin was followed by a rise in blood pressure, at the peak of which another sample of skeletal muscle was taken. After waiting for 30 minutes, 0.5 cc. per kg. of a 1:1000 solution of nitroglycerin was injected intravenously and when the blood pressure had fallen below the original resting level the last biopsy of skeletal muscle was taken. All specimens were fixed for the same period and prepared for histological examination in the same manner. Sections were cut at intervals of about 0.5 mm. from each block of tissue and 50 arterioles ranging from 18 to 72 μ in external diameter were measured in each specimen. The results of these measurements are recorded in Table VII.

To determine whether the observed differences were significant or not, 7 samples of skeletal muscle were taken at the same time from the shoulder of 1 dog and the samples were prepared for microscopic examination in the same manner. Fifty arterioles (18 to 72 μ in external diameter) in each sample were measured as in the preceding experiment and the measurements were recorded in Table VIII. It was seen that the variations in mean external diameter, mean internal diameter and mean wall to lumen ratio, associated with differences in the physiological state of the vascular system, were no greater than those observed in the samples taken under uniform physiological conditions. Although this experiment does not prove that variations in the

TABLE VII

A Comparison of the Mean External Diameter, the Mean Internal Diameter and the Mean Wall to Lumen Ratio of Arterioles in Samples of Skeletal Muscle (50 Arterioles Ranging Between 18 and 72 μ in External Diameter in Each Sample) in Different States of Arterial Hyper- and Hypotension from Each of 4 Dogs

Dog No.	Experimental conditions	Systolic blood pressure	Source of muscle	Mean external diameter of 50 arterioles	Mean internal diameter of 50 arterioles	Mean wall to lumen ratio of 50 arterioles
		mm. Hg.		μ	μ	
1	Resting	160 to 180	Shoulder	32.4	14.8	1 to 1.7
	After adrenalin	270	"	35.3	15.5	1 to 1.6
	After nitroglycerin	140	"	37.6	17.3	1 to 1.7
2	Resting	150 to 170	"	29.5	15.8	1 to 2.3
	After adrenalin	240	"	29.2	15.1	1 to 2.1
	After nitroglycerin	120	"	29.9	15.1	1 to 2.0
3	Resting	160 to 170	Thigh	29.3	10.3	1 to 1.1
	After adrenalin	300 +	"	27.7	8.4	1 to 0.9
	After nitroglycerin	150	"	27.4	10.3	1 to 1.2
4	Resting	160 to 170	"	28.1	10.6	1 to 1.2
	After adrenalin	280	"	27.4	10.6	1 to 1.3
	After nitroglycerin	140	"	31.9	12.3	1 to 1.3

physiological state of vessels do not influence their size in microscopic preparations, yet it does indicate that anatomical variations, together with errors in mensuration under controlled conditions, account for as great differences as were observed in varying physiological states.

TABLE VIII

A Comparison of the Mean External Diameter, Mean Internal Diameter and Mean Wall to Lumen Ratio of Arterioles in Each of 7 Samples (50 Arterioles Ranging Between 18 and 72 μ in External Diameter in Each Sample) Taken at the Same Time and Subjected to the Same Methods of Tissue Preparation From the Shoulder Muscle of a Dog

Sample	Mean external diameter	Mean internal diameter	Mean wall to lumen ratio
	μ	μ	
1	30.2	16.2	1 to 2.3
2	27.4	14.8	1 to 2.3
3	26.3	13.3	1 to 2.0
4	27.0	13.7	1 to 2.1
5	25.9	12.6	1 to 1.9
6	28.1	13.7	1 to 1.9
7	28.8	16.9	1 to 2.8

PATHOGENESIS OF ARTERIOLAR SCLEROSIS

Impressions as to the pathogenesis of the various types of arteriolar sclerosis based on a statistical and morphological study of the disease were obviously inferential but certain relations and general trends deserve consideration.

Of perhaps the greatest significance is the fact that no type of chronic arteriolar disease was of and by itself pathognomonic of hypertension. Every type was found in persons known to have had normal blood pressures.

Intimal hyalinization appeared to be the arteriolar counterpart of simple arteriosclerosis (Plate 98), was essentially an aging phenomenon which developed precociously in some individuals and was seen most frequently in the abdominal organs supplied by relatively large short branches of the aorta (spleen, kidney,

pancreas and adrenals) as well as in the brain, spinal cord and eye. It was seen with greater frequency and severity in hypertensives than in non-hypertensives and in hypertensive individuals extended into vascular beds where its occurrence in non-hypertensives was very infrequent (liver and gastro-intestinal tract). Since intimal hyalinization appeared to be a simple "wear and tear" type of tissue reaction, and since it was reasonable to assume that chronic hypertension might augment arteriolar "wear and tear," it is not unreasonable to expect the lesion to be more widely distributed and more severe in hypertensives than in non-hypertensives. It is also possible that the arteriolar changes secondary to chronic hypertension frequently effect such widespread organic reduction in lumen caliber that the severity of the hypertension is increased, thus establishing a vicious circle. There was, however, no proof that hypertension initiated intimal hyalinization. The development of visible arteriolar disease in the retinal vessels in the course of essential hypertension and the later development of renal failure due to advanced nephrosclerosis do not necessarily indicate that these vascular changes are due to the high blood pressure. They may as well represent the further extension of a primary morbid process which causes hypertension. Neither does recovery from a state of chronic hypertension indicate that the disease is primarily functional rather than organic. Certainly organic vascular disease is not necessarily an irreversible process. The possibility that high blood pressure causes intimal hyalinization cannot be denied, but there is no conclusive evidence that such is the case and it is known that intimal hyalinization does occur quite independently of hypertension.

Another possible explanation for the increased severity of intimal hyalinization in hypertension is that only the more severe forms of arteriolar disease may in some way have been responsible for hypertension. Still another possible explanation is that the factors (humoral or reflex) that cause the arteriolar spasm that produces the increased peripheral resistance may also injure vessels with resulting intimal degeneration.

Intimal proliferation (Plate 101) was seen under three circumstances: (1) it was seen very frequently in association with chronic inflammation and apparently represented the characteristic vascular response to chronic inflammation in hypertensive

as well as non-hypertensive individuals; (2) it constituted a secondary adaptive change in arterioles supplying tissues that had undergone parenchymatous atrophy with a consequently diminished capillary bed and diminished blood flow (ovary and uterus); and (3) it was seen commonly as an independent and apparently primary morbid process most frequently in the kidney and occasionally in other tissues (pancreas, adrenal capsules, gall bladder, seminal vesicles, spleen, gastro-intestinal tract, and eyes of hypertensives.

The pathogenesis of the intimal hyperplasia in the first two of the above considered conditions seems to be primary exudative or non-exudative arteriolitis or arteriolar involution. The third consideration is best exemplified by the obliterating arteriolar intimal hyperplasia of nephrosclerosis in chronic hypertension and may be the result of inflammation, involution, neither or both. It is possible or even probable that this is a form of arteriolitis, occurring either as an augmented type of "wear and tear" arteriolar degeneration (Herxheimer) or as a primary independent arteriolar inflammation (Fahr). It is also possible that some or all of the arteriolar endothelial proliferation in the contracted kidney represents arteriolar involution secondary to obstruction proximal to it by spasm or distal to it by glomerular contraction (Volhard, Fishberg). It has been suggested by Moschcowitz that the endothelial proliferation is caused by the hypertension and in support of this theory he cites the endothelial hyperplasia of pulmonary arterioles in cases of mitral stenosis. The analogy is obviously not entirely applicable, inasmuch as in mitral stenosis there is, in addition to pulmonary arterial hypertension, stasis and cardiovascular inflammation.

Medial hypertrophy and degeneration uncomplicated by intimal thickening (Plates 99 and 100) were seen with greater frequency and severity in hypertensives than in non-hypertensives. The change appeared to be structurally analogous to the hypertrophy of any other hollow muscular structure. It is not known that arteriolar dilatation occurs in life as a result of high blood pressure, but it is presumed that arteriolar constriction does occur. Increased peripheral resistance to blood flow due to vasoconstriction is a *sine qua non* of chronic hypertension. Arteriolar dilatation is then the apparent antithesis of the vascular state of hypertension.

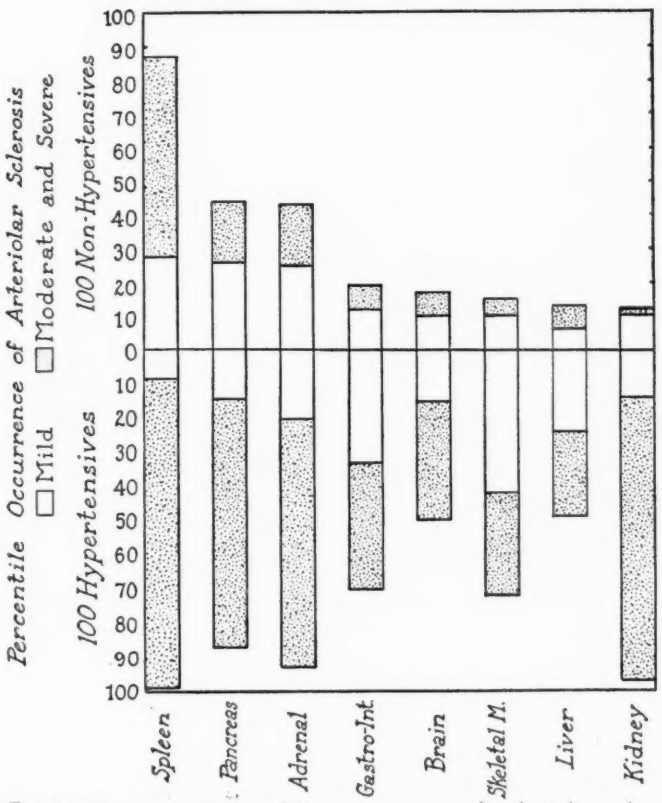
The microscopic changes in this type of arteriolar disease were similar to those occurring in other hollow muscular structures subjected to increased internal expansile pressure. It is not known whether vasoconstriction in hypertension is segmental or general, but if it is segmental it can be understood how dilatation might take place proximal to contracted segments or, as suggested by Volhard, may antedate the distal arteriolar spasm which results in the abnormal peripheral resistance. If medial disease is in some circumstances primary, it is conceivable that wall stretching might occur even with normal blood pressure and medial hypertrophy result. Although no conclusive evidence has been offered in support of the view that this medial change is secondary to stretching, such an explanation appears tenable.

In summary it may be said that the two most prominent and frequently occurring types of arteriolar disease, intimal hyalinization and intimal hyperplasia, appear to be primary pathological processes. The former is an aging phenomenon and the latter appears to be either an accelerated type of the former or an independent, non-exudative, productive arteriolitis. Medial hypertrophy appears on anatomical grounds to be a secondary phenomenon, probably related to stretching of vessels by the intravascular bursting tension. The medial degeneration may be in part primary, leading to smooth muscle stretching in non-hypertensive as well as in hypertensive individuals. Severe forms of arteriolar medial disease were seen only in persons with chronic hypertension.

Correlation of Arteriolar Sclerosis and Chronic Hypertension

In the foregoing review of the histological types, relative severity and distribution of arteriolar sclerosis in hypertensive and non-hypertensive individuals, two very pertinent facts have emerged. One is the high degree of correlation between renal arteriolar sclerosis and hypertension, and the other is the extreme rarity of more than a very mild degree of renal arteriolar sclerosis in non-hypertensive individuals (Text-Fig. 7). It should be recalled that the tissues from these 200 cases were examined objectively. Renal arteriolar sclerosis was recorded as being present in 109 persons. In 97 of these 109 cases of nephrosclerosis an

examination of the clinical records, corroborated by heart weights, revealed chronic hypertension to have been present. This permits the conclusion that at the time of death almost every chronic



Text Fig. 7. A comparison of the occurrence of arteriolar sclerosis in the various organs and tissues of 100 hypertensive and 100 non-hypertensive individuals.

hypertensive (97 per cent) has renal arteriolar sclerosis in some degree, that few non-hypertensives (12 per cent) have any renal arteriolar sclerosis, and that in only 2 per cent of the non-hypertensives is there more than mild arteriolar nephrosclerosis (see Text-Fig. 7). No comparable correlation could be found in the

case of any other organ or tissue. Although in hypertensives the arterioles of the spleen were frequently as sclerotic as those of the kidney, they were also found to be diseased in 86 per cent of the non-hypertensives. With the exception of the kidney, correlation of arteriolar sclerosis and hypertension is either not constant enough to indicate a significant relation or the organ or tissue shows arteriolar sclerosis so frequently in non-hypertensive persons that the significance of the positive correlation is destroyed.

Inquiry should next be directed at the causal relation of renal arteriolar sclerosis to hypertension. The two obvious possibilities are: (1) that hypertension causes the sclerosis of renal arterioles; and (2) that renal arteriolar sclerosis causes the hypertension.

The first explanation would attribute to the renal arterioles an extremely high degree of selective vulnerability to high blood pressure, since they were affected in almost every case of hypertension but were rarely diseased in any other circumstances. At the same time it would be necessary to attribute to the renal arterioles a high degree of resistance to every injury except the mechanical damage incident to elevated blood pressure. Arterioles in many parts of the body were found to be severely diseased in hypertensives, but with the exception of the kidney the same relative susceptibility of tissues to arteriolar sclerosis was seen in hypertensive and non-hypertensive individuals.

The second possible explanation, namely, that renal arteriolar sclerosis causes hypertension, is far more tenable. Arteriolar sclerosis occurs as a primary pathological change. It is entirely reasonable to assume that this primary vascular disease may affect the renal arterioles as well as those in the spleen, pancreas, adrenals and other tissues. When the renal arterioles become sclerotic, hypertension is almost invariably present. This conclusion is based on objective evidence in human postmortem material and is of especial significance because of the close correlation it establishes between essential hypertension in man and the experimental production of chronic hypertension in dogs by Goldblatt and collaborators. Goldblatt's original observations, subsequently confirmed by Page, Elaut, Wood and Cash, Collins, Harrison, Blalock and Mason, and Prinzmetal and Friedman, that reduction in blood flow through the main renal arteries of dogs by the use of clamps invariably led to chronic hypertension, is in entire accord

with the pathological anatomical findings in chronic hypertension in man. The renal arteriolar sclerosis in man appears to have the same functional effect as the silver clamp around the main renal arteries in dogs.

The fact that renal arteriolar sclerosis does in some instances occur without an associated hypertension in no way detracts from the major hypothesis. It is obvious that the reduction of blood flow through the kidney is not the entire mechanism involved in the production of chronic hypertension. Some humoral or reflex influence must lead to the generalized spasm of arterioles which makes hypertension possible. Even though the renal vascular disease with resulting reduction of blood flow through the kidneys is present, it is conceivable that other conditions are not favorable for the development of hypertension. A certain degree of myocardial competence and a sustained resistance to blood flow through the peripheral vessels is obviously necessary for the maintenance of high blood pressure. It is possible that as a result of natural causes or surgical intervention the functional response (hypertension) to reduced blood flow through the kidneys (arterial or arteriolar nephrosclerosis, chronic diffuse glomerulonephritis, congenital polycystic renal disease, and so on) may in some instances be inhibited. Furthermore, it is not possible to translate accurately structural change into functional effect. What appears morphologically to be mild arteriolar disease may in reality account for considerable reduction in renal blood flow, and the converse is equally true.

In this consideration of the correlation of chronic hypertension and the distribution of arteriolar sclerosis, attention must be paid to the 3 cases of chronic hypertension in which there was no significant degree of renal arteriolar sclerosis (see Text-Fig. 7).

The 1st case was a female, 54 years of age, who died of cardiac tamponade following the rupture of an aortic aneurysm. Blood pressure determinations prior to her terminal illness varied about the figure of 220/120. The heart weight was 450 gm. and there was severe renal arteriosclerosis.

The 2nd case was that of a female, 75 years of age, who died of pulmonary embolism following a period of mild cardiac decompensation. Her mean blood pressure during the period of observation was 160/90 and the heart weight was 680 gm. with-

out significant concomitant heart disease. At autopsy severe renal arteriosclerosis was observed and there were obstructing annular plaques around the aortic ostia of the renal arteries.

The 3rd case was a male, 60 years of age, who died of cardiac rupture through a myocardial infarct. Preceding the terminal infarct, blood pressure of 190/110 was observed. The heart weighed 500 gm. and there was a severe renal arteriosclerosis, with scattered coarse scars throughout both kidneys.

All 3 had renal arteriosclerosis of sufficient severity to constitute an independent postmortem diagnosis. If it be assumed that these were undoubted cases of chronic hypertension, their hypertension is susceptible to the same explanation as might be offered in the case of renal arteriolar sclerosis. The vascular changes are consistent with, although not definitely confirmatory of, reduced blood flow through the kidneys.

Types of Chronic Hypertension

In these 100 cases of chronic hypertension there were neither clinical nor pathological criteria for establishing definite subgroups. Certainly there were some cases in which the disease ran a more acute course than in others, and such disease was found more frequently in persons under 45 years of age and usually terminated in death from renal insufficiency. This vaguely defined group has been spoken of as "malignant hypertension" in contrast to "benign hypertension" which affected an older group, progressed more slowly and characteristically terminated in heart failure or cerebral hemorrhage if not interrupted prematurely by accident or unrelated illness.

In reviewing the clinical data it appeared to be much easier to identify cases of chronic hypertension as malignant or benign in retrospect than it was to prognosticate the course of the disease from its beginning. There appeared to be no way of predicting the speed at which the disease would progress except on the basis of the rate of the cardiac or renal deterioration that had already occurred. Certainly the height and fixation of the blood pressure provided no constant index of the rapidity with which the disease would progress, nor did observation of the severity of vascular changes by ophthalmoscopic examination, except as they indicated the degree to which the disease had already progressed. Measure-

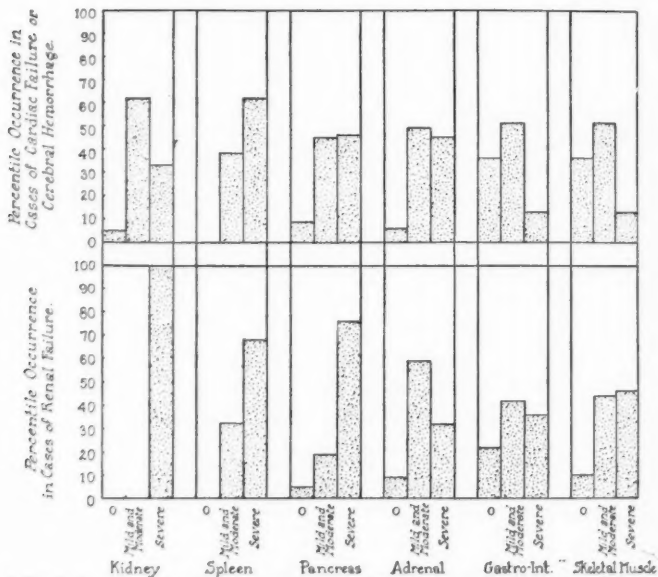
ments of a large number of arterioles in biopsy of pectoral muscle did not make it possible even to identify the presence of hypertension in more than half of the cases in which high blood pressure was present for a long time and provided no useful information in distinguishing between the "benign" and "malignant" form of the disease.

Certain group differences in the pathological anatomy of the so-called "benign" and "maligant" hypertension were apparent. The essential difference appeared to lie in the rapidity with which the vascular disease progressed in the kidney. If the arteriolar nephrosclerosis progressed with sufficient rapidity, renal insufficiency developed before the duration of the hypertension was sufficient to determine death from heart failure or cerebral hemorrhage. However, a number of examples were found of a rapidly progressing renal vascular disease in which heart failure or cerebral hemorrhage interrupted what might be properly regarded as the natural termination of the disease by renal failure. As a rule such cases were of older persons with severe coronary or cerebral arterial sclerosis.

In the material on which this investigation was based, in all the cases of so-called "malignant" hypertension severe generalized arteriolar nephrosclerosis was present. Not only was there occlusive intimal hyalinization of the afferent arterioles, but the small arteries from which they were derived were the seat of obliterating endothelial proliferation. A wide variety of acute degenerative and inflammatory changes was observed to occur in vessels already chronically diseased. These changes have been discussed in detail by Fahr, Klemperer and Otani, and Schurmann and MacMahon. An examination of Text-Fig. 8 suggests that on anatomical grounds this type of hypertension does not differ qualitatively from "benign" hypertension but is an accelerated and more severe type of vascular disease than is seen in the benign form. This conclusion is in essential agreement with the opinion of Herxheimer and Löhlein. This was especially true in the kidney, for in 100 per cent of the cases with death from renal failure, the vascular disease had been graded as severe.* In every organ and

* This does not imply that severe vascular changes are regularly seen in all cases of death from renal failure. In the 100 cases of chronic hypertension on which this study was based, all instances of death from renal insufficiency were by chance in cases of severe arteriolar nephrosclerosis.

tissue examined the arteriolar disease occurred with greater frequency and severity in the "malignant" than in the "benign" types of hypertension. Exclusive of the acute secondary arteriolar lesions, there was no type of vascular change in any tissue or organ that provided absolute grounds for distinguishing between malignant and benign hypertension. Although 100 per cent of the kidneys in cases of malignant hypertension showed severe



Text Fig. 8. A comparison of the occurrence, distribution and severity of arteriolar sclerosis in chronic hypertensives dying of renal failure and those dying of cardiac failure or cerebral hemorrhage.

arteriolar disease, equally severe renal arteriolar sclerosis was observed in 23 per cent of the cases of benign hypertension. Although 41 per cent of the cases of malignant hypertension had severe arteriolar lesions in skeletal muscles, 10 per cent of the benign hypertensives had equally severe arteriolar lesions in the skeletal muscles.

In both forms of hypertension the vascular disease was characteristically generalized and was represented by the same histological types of arteriolar lesions which were more severe and as a rule more widely distributed in individuals dying of renal

TABLE IX

The Number of Patent Renal Glomeruli in Various Types of Chronic Hypertension *

Death due to renal failure					Death due to cardiac failure or cerebral hemorrhage				
Age	Sex	Heart weight	Renal arteriolar sclerosis	Total No. of patent glomeruli	Age	Sex	Heart weight	Renal arteriolar sclerosis	Total No. of patent glomeruli
yrs. 55	F	*gm.* Enlarged	Severe	*thousands* 101	*yrs.* 67	M	*gm.* 780	Severe	*thousands* 378
27	F	450	Severe	169	64	M	500	Severe	452
58	M	460	Severe	240	38	F	510	Moderate	490
48	F	Enlarged	Severe	243	70	M	550	Severe	645
35	M	650	Severe	254	46	M	875	Moderate	703
41	M	470	Severe	269	50	M	650	Moderate	749
49	F	430	Severe	386	62	M	500	Moderate	834
44	M	600	Severe	422	41	F	830	Severe	908
49	F	500	Severe	412	70	M	700	Mild	908
40	F	450	Severe	459	57	F	600	Moderate	972
58	M	740	Severe	510	52	M	600	Moderate	988
40	F	540	Severe	654	40	M	600	Mild	980

* The authors wish to express their appreciation to Dr. J. M. Hayman, Jr., for the glomerular counts.

insufficiency than in those dying of heart failure or cerebral hemorrhage.

Quantitative estimations of the degree of renal destruction by means of glomerular counts corroborated the impression gained from the histological examination and from the clinical course of the disease. Glomerular counts by Vimtrup's method were made on the kidneys of 24 chronic hypertensives (Table IX).

These 24 were comprised of two groups of 12 cases each. The mean age of the first group at the time of death was 45 years and of the second group, 55 years. Hypertension was generally of shorter duration in the first group in which death was the result of renal insufficiency. The second group had longer records of known chronic hypertension and death was due either to heart failure or cerebral hemorrhage. There were no significant differences in the degree of hypertension in the two groups. Cardiac hypertrophy was more pronounced in the group dying of heart failure or cerebral hemorrhage than in the group dying of renal insufficiency.

There was a striking difference in the numbers of patent glomeruli per kidney in the two groups. It has been shown by Moritz and Hayman that 1,250,000 is the approximate number of patent glomeruli in the normal kidney. In most of the individuals dead of renal failure there was a pronounced reduction in the number of injectible glomeruli, as compared with those dead of heart failure or cerebral hemorrhage. With full regard for the fact that a patent glomerulus is not necessarily a normal one, it is apparent that although the degree and duration of hypertension seem to be independent of the actual amount of renal destruction, death from renal insufficiency is associated with a measurable increase in the amount of glomerular destruction.

Conclusions

General Characteristics of Essential Hypertension

The mortality and probably the morbidity of essential hypertension was greater in blacks than in whites and the mean age at the time of death was lower in blacks than in whites.

The proportion of cases of essential hypertension with death

from renal insufficiency, heart failure and cerebral hemorrhage was the same in blacks as in whites.

There were no differences between males and females in mortality, cause of death or age at time of death from chronic hypertension.

The mean age at the time of death was lower in individuals dying of renal failure than in those dying of heart failure or cerebral hemorrhage.

In almost half of all cases of essential hypertension with death from heart failure, postmortem examination disclosed occlusive coronary disease.

Histological Types of Chronic Arteriolar Disease

The three principal histological types of chronic arteriolar disease included: (1) intimal hyalinization; (2) medial hypertrophy and degeneration; and (3) intimal proliferation.

All three types, separately and in combination, were found in non-hypertensives as well as in hypertensives. Although no direct information was available as to the etiology of the three types there was indirect evidence in support of the view:

1. That intimal hyalinization is the arteriolar counterpart of simple arteriosclerosis, is essentially a degenerative change, and becomes more widespread and severe with advancing age.

2. That medial hypertrophy and degeneration are changes resembling those following distention of any hollow muscular structure, and although medial degeneration may be primary in some instances, yet the medial hypertrophy is probably secondary to stretching and was seen with greater frequency and severity in hypertensives than in non-hypertensives.

3. That endothelial hyperplasia with increase in elastic tissue and secondary degenerative changes was seen: commonly in association with inflammation where it was properly called endarteritis obliterans; commonly as an adaptive involutional change in vessels whose capillary beds have been reduced by parenchymatous atrophy; and commonly in cases of hypertension where it may have represented a primary vascular inflammation or an accelerated form of arteriolar sclerosis.

Measurements of Arterioles (Medial Hypertrophy)

Neither the mean wall to lumen ratio, the mean external nor the mean internal diameter of large samples of arterioles from the skeletal muscle were useful in distinguishing between hypertensive and non-hypertensive individuals. Relative arteriolar wall thickening was a group characteristic for the hypertensives and, so far as histological preparations of tissues were concerned, the thickening resulted in an increase in external diameter rather than a decrease in the lumen diameter. The most useful numerical value in distinguishing hypertensives from non-hypertensives was the mean external diameter of arterioles within a fixed size range. It was possible to identify 48 per cent of the hypertensives by measurements that were in excess of those seen in any non-hypertensives, and it was possible to identify 16 per cent of the control cases by measurements lower than were observed in any of the hypertensives. Measurements of arterioles were not useful in distinguishing between various types of essential hypertension.

Neither the physiological state of the vascular system at the time the specimens were obtained for microscopic examination nor variations in the methods used in preparing tissues for microscopic examination led to changes in arteriolar dimensions that were greater than those resulting from anatomical variations or errors in mensuration under controlled conditions.

Correlation of Arteriolar Disease and Chronic Hypertension

The objective examination of arterioles in all parts of the body of 100 control cases and 100 cases of chronic hypertension disclosed only one situation in which the presence of arteriolar sclerosis was almost invariably associated with hypertension and where the absence of arteriolar sclerosis almost invariably betokened an absence of high blood pressure. This was in the kidneys. Renal arteriolar sclerosis was present in 109 of the 200 cases studied, and 97 of these 109 proved to be cases of chronic hypertension. No comparable correlation could be found in any other organ or tissue.

It was felt that these facts, together with the information gained from a study of the histological characteristics of arteriolar disease in hypertensive and non-hypertensive individuals, supported

the conclusion that renal arteriolar sclerosis is the most common cause of chronic hypertension. This conclusion is in accord with the recent demonstration by Goldblatt that chronic hypertension is regularly produced in dogs and monkeys by reducing the blood flow through the kidneys (renal ischemia). The effect of the renal arteriolar sclerosis in human hypertension appears to be the functional analogue of the renal arterial clamp in experimental hypertension. In both instances hypertension appears to be produced by reduction in renal blood flow which does not necessarily lead to a sufficient degree of ischemia to impair renal function measurably.

It is concluded that the only significant site of arteriolar sclerosis so far as the causation of hypertension is concerned is the kidney.

Types of Chronic Hypertension

The material on which this investigation was based did not include cases in which the hypertension could be attributed to chronic nephritis, congenital polycystic renal disease, urinary obstruction, obesity, hyperthyroidism, aortic insufficiency, aortic coarctation, pituitary or adrenal tumors, or arteriovenous aneurysm. Such causes of hypertension were not excluded deliberately but were simply not encountered in the selection of cases by the criteria outlined in the early part of this report. A survey of the rate of progress of the disease, the mean age at the time of death and the cause of death disclosed two groups which were best separated by the manner in which the disease terminated. One group died of renal insufficiency and for several reasons was most adequately described as "malignant" hypertension in contrast with a group that died of heart failure or cerebral hemorrhage which was designated as "benign" hypertension. The application of the terms "malignant" and "benign" denoted group characteristics applicable in retrospect rather than constituting useful terms in predicting the course of the disease in its early stages. The factor which determines that some cases of essential hypertension will run a more rapid course with early death from renal insufficiency does not appear to be the degree of the hypertension but rather the rate of renal destruction incident to the progressive character of the renal vascular disease. Although no correlation could be established between varying degrees of renal vascular disease and vary-

ing degrees of blood pressure elevation, there did appear to be a correlation between the amount of renal atrophy, as indicated by glomerular counts and the mode of death.

Note: The authors wish to express their indebtedness to Miss Theodora Bergsland for the colored illustrations.

BIBLIOGRAPHY

Andrus, Frank C. The relation of age and hypertension to the structure of the small arteries and arterioles in skeletal muscle. *Am. J. Path.*, 1936, **12**, 635–654.

Bell, E. T. Arteriosclerosis, Cowdry, Edmund V. The Macmillan Company, New York, 1933.

Bell, E. T., and Clawson, B. J. Primary (essential) hypertension; a study of four hundred and twenty cases. *Arch. Path.*, 1928, **5**, 939–1002.

Benninghoff, A. Handbuch der mikroskopischen Anatomie des Menschen, von Möllendorff, Wilhelm. J. Springer, Berlin, 1930, **6**, Pt. 1.

Boyd, William. The Pathology of Internal Diseases. Lea & Febiger, Philadelphia, 1935, Ed. 2.

Bright, R. Cases and observations illustrative of renal disease accompanied with the secretion of albuminous urine. *Guy's Hosp. Rep.*, 1836, **1**, 339–379.

Christian, Henry A., and O'Hare, James P. Nephritis. Oxford Medicine, Oxford University Press, New York, III, 772 (28).

Collins, Dean A. Hypertension from constriction of the arteries of denervated kidneys. *Am. J. Physiol.*, 1936, **116**, 616–621.

Cowdry, E. V. Arteriosclerosis. The Macmillan Company, New York, 1933.

Elaut, L. Hypertension artérielle chronique chez le chien par ischémie rénale. *Compt. rend. Soc. de biol.*, 1936, **122**, 126–127.

Evans, Geoffrey. A contribution to the study of arterio-sclerosis, with special reference to its relation to chronic renal disease. *Quart. J. Med.*, 1921, **14**, 215–282.

Ewald, C. A. Ueber die Veränderungen kleiner Gefässe bei Morbus Brightii und die darauf bezüglichen Theorien. *Virchows Arch. f. path. Anat.*, 1877, **71**, 453–499.

Fahr, Th. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O. J. Springer, Berlin, 1934, **6**.

Fishberg, Arthur M. Anatomic findings in essential hypertension. *Arch. Int. Med.*, 1925, **35**, 650–668.

Fishberg, Arthur M. Hypertension and Nephritis. Lea & Febiger, Philadelphia, 1935, Ed. 3.

Goldblatt, Harry. Studies on experimental hypertension. III. The production of persistent hypertension in monkeys (macaque) by renal ischemia. *J. Exper. Med.*, 1937, **65**, 671–675.

Goldblatt, Harry, Lynch, James, Hanzal, Ramon F., and Summerville, Ward W. Studies on experimental hypertension. I. The production of persistent elevation of systolic blood pressure by means of renal ischemia. *J. Exper. Med.*, 1934, **59**, 347–379.

Gull, Sir William W., and Sutton, Henry G. On the pathology of the morbid state commonly called chronic Bright's disease with contracted kidney ("arterio-capillary fibrosis"). *Med.-Chir. Tr. London*, 1872, **55**, 273–329.

Harrison, T. R., Blalock, A., and Mason, M. F. Effects on blood pressure of injection of kidney extracts of dogs with renal hypertension. *Proc. Soc. Exper. Biol. & Med.*, 1936, **35**, 38–40.

Hewlett, Albion Walter. The Pathological Physiology of Internal Diseases. Appleton and Company, New York, 1928.

Herxheimer. Niere und Hypertonie. *Verhandl. d. deutsch. path. Gesellsch.*, 1912, **15**, 211–216.

Johnson, George. Lectures on the pathology, diagnosis, and treatment of Bright's disease. Lecture III. Chronic Bright's disease. *Brit. M. J.*, 1873, 1, 161–165.

Johnson, George. I. On certain points in the anatomy and pathology of Bright's disease of the kidney. II. On the influence of the minute blood-vessels upon the circulation. *Med.-Chir. Tr. London*, 1868, **51**, 57–78.

Jores, Leonhard. Arterien. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O. J. Springer, Berlin, 1924, **2**, 608–786.

Jores, L. Über die Arteriosklerose der kleinen Organarterien und ihre Beziehungen zur Nephritis. *Virchows Arch. f. path. Anat.*, 1904, **178**, 367–406.

Karsner, H. T. Primary inflammation of arteries. *Ann. Int. Med.*, 1937, **11**, 164–174.

Karsner, H. T., and Bayless, F. Coronary arteries in rheumatic fever. *Am. Heart J.*, 1934, **9**, 557–585.

Keith, Norman M., Barker, Nelson W., and Kernohan, James W. Histologic studies of the arterioles in various types of hypertension. *Tr. A. Am. Physicians*, 1931, **46**, 66–70.

Kernohan, James W., Anderson, Edward W., and Keith, Norman M. The arterioles in cases of hypertension. *Arch. Int. Med.*, 1929, **44**, 395–423.

Klemperer, Paul, and Otani, Sadao. "Malignant nephrosclerosis" (Fahr). *Arch. Path.*, 1931, **11**, 60–117.

Löhlein, M. Zur Pathogenese der vasculären Schrumpfniere. *Med. Klin.*, 1916, **12**, 741–743.

Mahomed, Fred A. The etiology of Bright's disease and the prealbuminuric stage. *Med.-Chir. Tr. London*, 1874, **57**, 197–228.

Maximow, Alexander A., and Bloom, William. A Textbook of Histology. W. B. Saunders Company, Philadelphia, 1935, Ed. 2.

Moritz, Alan R., and Beck, Claude S. The production of a collateral circulation to the heart. II. Pathological anatomical study. *Am. Heart J.*, 1935, **10**, 874–880.

Moritz, Alan R., and Hayman, J. M. The disappearance of glomeruli in chronic kidney disease. *Am. J. Path.*, 1934, **10**, 505–518.

Moschcowitz, Eli. The pathology of hypertension. *J. A. M. A.*, 1922, **79**, 1196–1200.

Page, I. H. Relationship of extrinsic renal nerves to origin of experimental hypertension. *Am. J. Physiol.*, 1935, **112**, 166–171.

Parker, Frederic, Jr., and Weiss, Soma. The nature and significance of the structural changes in the lungs in mitral stenosis. *Am. J. Path.*, 1936, **12**, 573–598.

Prinzmetal, Myron, and Friedman, Ben. Pressor effects of kidney extracts from patients and dogs with hypertension. *Proc. Soc. Exper. Biol. & Med.*, 1936, **35**, 122–124.

Schürmann, P., and MacMahon, H. E. Die maligne Nephrosklerose, zugleich ein Beitrag zur Frage der Bedeutung der Blutgewebsschranke. *Virchows Arch. f. path. Anat.*, 1933, **291**, 47–218.

Scott, R. W., Seecof, David P., and Hill, Albert A. Arteriolar lesions of skeletal muscle in hypertension. *Tr. A. Am. Physicians*, 1933, **48**, 283–288.

Vimtrup, B. J. On the number, shape, structure, and surface area of the glomeruli in the kidneys of man and mammals. *Am. J. Anat.*, 1928, **41**, 123–151.

Volhard, Franz. The Kidney in Health and Disease, Berglund, Hilding, Medes, Grace, Huber, G. Carl, Longcope, Warfield T., Richards, Alfred N. Lea & Febiger, Philadelphia, 1935.

Wood, J. Edwin, Jr., and Cash, James R. Experimental hypertension — Observations on sustained elevation of systolic and diastolic blood pressure in dogs. *J. Clin. Investigation*, 1936, **15**, 543–557.

DESCRIPTION OF PLATES

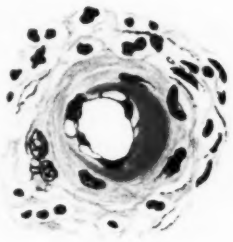
PLATE 98

Intimal Hyalinization

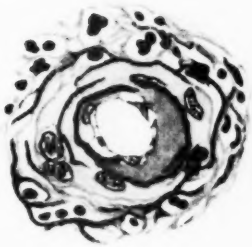
FIG. 1. Arteriole showing plaque of intimal hyalin.
a = Hematoxylin-eosin stain.
b = Combination of van Gieson's and Weigert's elastic methods on same section. × 500.

FIG. 2. Annular form of intimal hyalinization showing beginning degeneration of internal elastic lamella incorporated within the hyalin.
a = Hematoxylin-eosin stain.
b = Combination of van Gieson's and Weigert's elastic methods on same section. × 500.

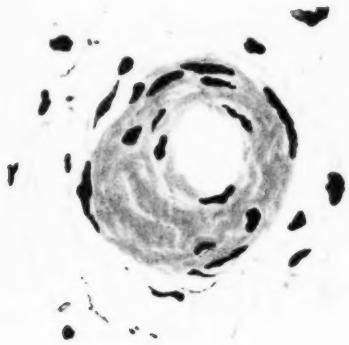
FIG. 3. Intimal hyalinization in a dilated, thin-walled vessel.
a = Van Gieson's method.
b = Weigert's elastic method on same section. × 500.



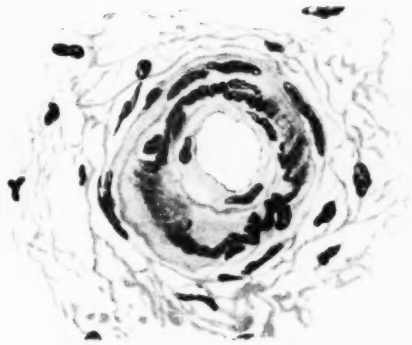
1a



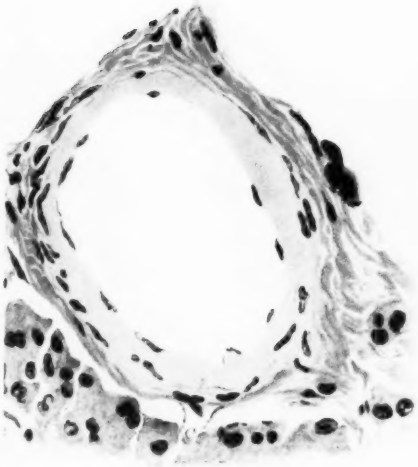
1b



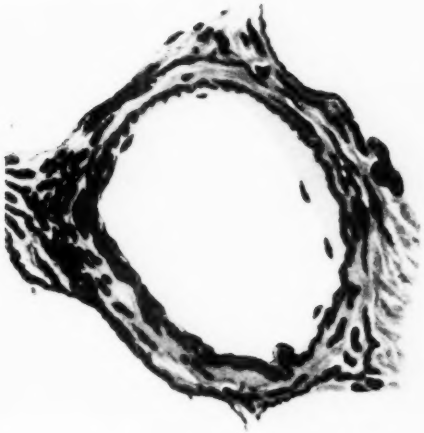
2a



2b



3a



3b

Moritz and Oldt

Arteriolar Sclerosis

UNIV. OF MICH.

PLATE 99

Medial Hypertrophy

FIG. 4. Normal arteriole showing increased wall thickness as the result of pronounced postmortem contraction.

a = Hematoxylin-eosin stain.

b = Combination of van Gieson's and Weigert's elastic methods on same section. × 500.

FIG. 5. Hypertrophy of media.

a = Hematoxylin-eosin stain.

b = Combination of van Gieson's and Weigert's elastic methods on same section. × 500.

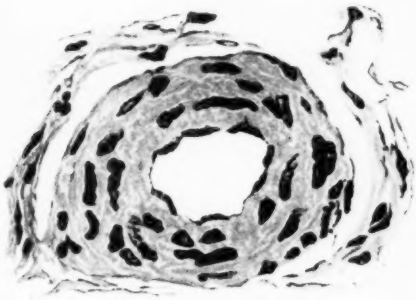
FIG. 6. Collagenous degeneration and medial hypertrophy.

a = Hematoxylin-eosin stain.

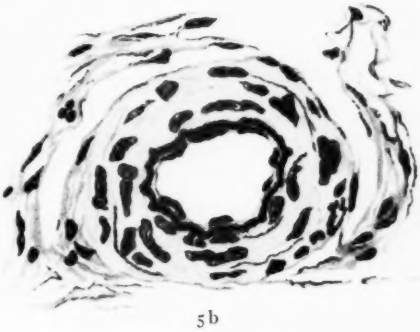
b = Combination of van Gieson's and Weigert's elastic methods on same section. × 500.

4a

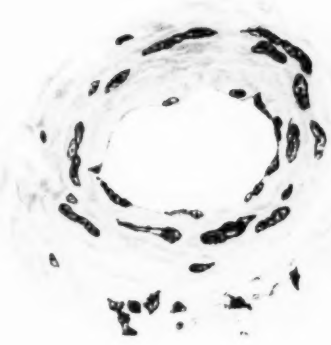
4b



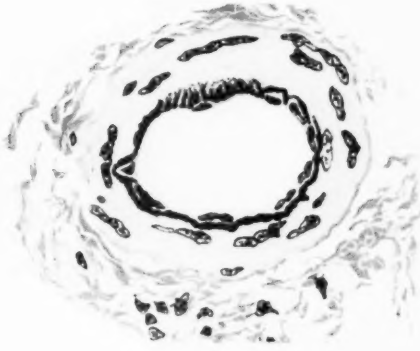
5a



5b



6a



6b

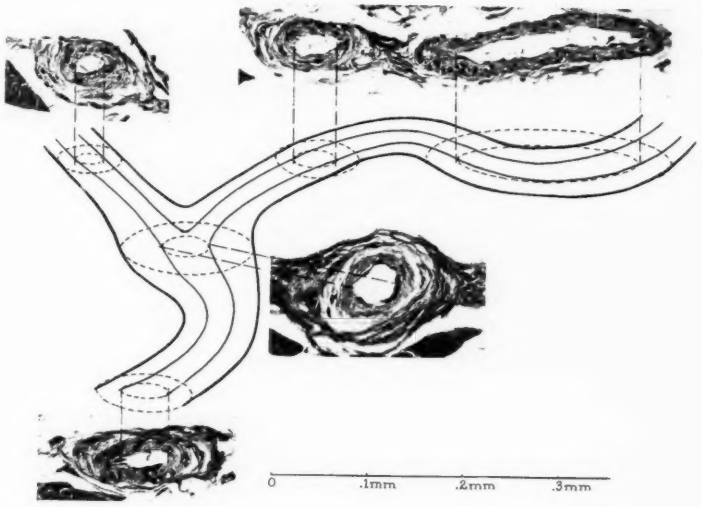
Moritz and Oldt Arteriolar Sclerosis

UNIV. OF MICH.

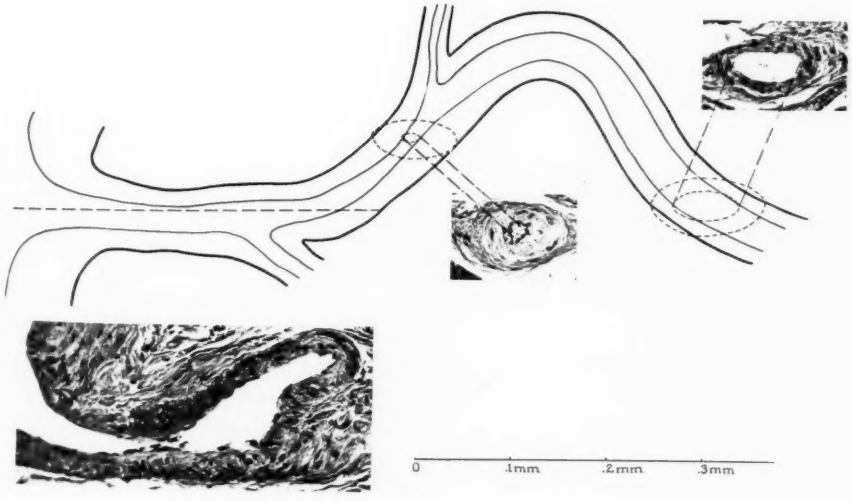
PLATE 100

FIG. 7. Reconstruction of an arteriole from the pectoral muscle of an individual with chronic hypertension showing the non-uniform character of the vascular disease in different segments of the same vessel.

FIG. 8. Reconstruction of an arteriole from the pectoral muscle of an individual with chronic hypertension showing the non-uniform character of the vascular disease in different segments of the same vessel.



7



8

UNIV. OF MICH.

PLATE 101

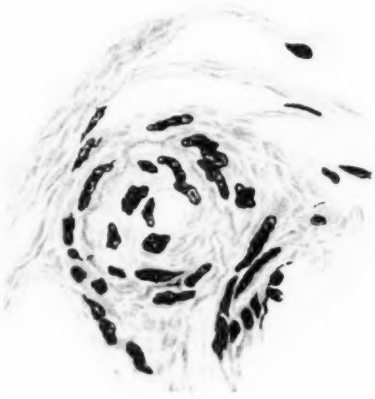
Endothelial Hyperplasia

FIG. 9. Endothelial hyperplasia within an intact internal elastic lamella with pronounced intimal thickening and reduction in lumen caliber.

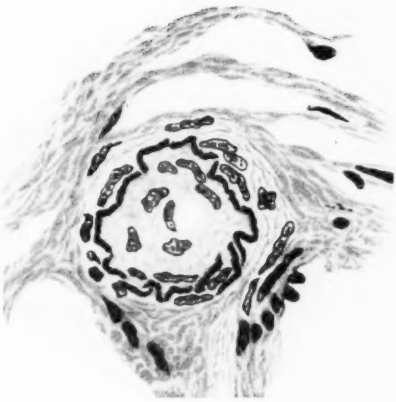
a = Hematoxylin-eosin stain.
b = Combination of van Gieson's and Weigert's elastic methods on same section. × 500.

FIG. 10. Endothelial hyperplasia associated with reduplication of internal elastic lamella and degeneration of old and new elastic tissue.

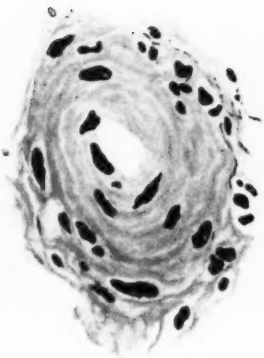
a = Hematoxylin-eosin stain.
b = Van Gieson's method.
c = Weigert's elastic method. × 500.



9a



9b



10a

10b

10c

Moritz and Oldt

Arteriolar Sclerosis

INTRANUCLEAR INCLUSION BODIES IN THE TISSUE REACTIONS PRODUCED BY INJECTIONS OF CERTAIN FOREIGN SUBSTANCES *

PETER K. OLITSKY, M.D., AND CARL G. HARFORD, M.D.

(From the Laboratories of the Rockefeller Institute for Medical Research, New York, N. Y.)

In studies on prevention of experimental equine encephalomyelitis in guinea pigs by means of virus adsorbed on aluminum hydroxide Cox and Olitsky [1, 2] observed in the phagocytic mononuclear and giant cells of the induced subcutaneous nodules intranuclear inclusion bodies characteristic of encephalomyelitis virus infection. When the chemical alone, free from virus, was introduced under the skin of guinea pigs, similar inclusions were seen in the resulting foreign body reaction. This finding gave a point of departure for the present investigation in which an attempt was made to study further the nature and significance of the nuclear changes brought about through the action of non-virus material.

Intranuclear inclusion bodies are found as characteristic changes in the lesions of many virus diseases.[3, 4] In addition they have often been observed in tissues in which no search was made for the presence of virus although infection with virus was strongly suspected.[5, 6] Cowdry,[3, 4, 7] however, has stated that such structures are not necessarily the result of virus action and has held to the possibility that they may be produced experimentally by other means. A number of investigators, using non-infective materials, have produced bodies resembling the inclusions. It is noteworthy that in none of these procedures have attempts been made to eliminate the presence of virus occurring spontaneously in the animal tissues. (Summaries of and references to such reports are given in several articles.[4, 8-12])

A wide variety of methods and materials was used in these recorded instances. Bodies which investigators or their later commentators believed to resemble virus inclusions were found in tissues moistened with ammonium chloride followed by applica-

* Received for publication May 11, 1937.

tions of direct electric current; in nerve cells repeatedly stimulated by electric current; in ganglionic cells soaked in hypertonic sodium and calcium chlorides; in rabbit corneas injected with bacterial toxins, and in pad tissues injured by heat or acid. Among other materials used were: hypertonic solutions of glucose, sodium chloride, and bicarbonate; bismuth, lead, arsenic, morphine, strychnine, salyrgan, exotoxins, and so on, given to animals by various routes of inoculation.

In certain of these reported cases the nuclear changes might be regarded as not conforming with inclusions typical of virus infection. In some the entire nucleus was reported as shrunken [13]; in others, suspected bodies could well have been degenerated nucleoli which stained pink instead of blue [12]; in still others the basophilic chromatin was clumped about the nucleolus,[14] but in the remainder some resemblance to virus inclusions was apparent; for example, certain ones produced by Lee [15] with salyrgan were, in Cowdry's opinion,[4] indistinguishable from structures produced by viruses. As already mentioned, however, in none of these cases have attempts been made to exclude the possibility of a preexisting or concomitant virus as the causal factor in the production of the structures.

The following criteria are generally considered as the characteristic features of true virus inclusions. Their morphology must, of course, be based on studies of structures found only in virus-containing tissues; that of bodies which have no proved relationship with any virus is not applicable. By many investigations on undoubted virus inclusions it has evolved that no single characteristic feature is invariably found but that three particular properties are of outstanding importance, namely, (1) acidophilic staining, (2) halo formation, and (3) margination of chromatin with or without displacement of nucleoli. Cowdry [7, 16] has attempted to define the nuclear inclusions more precisely and to obtain clues of their chemical constitution by the use of histochemical reactions. Most noteworthy of these are the Macallum test for masked iron and the Feulgen reaction for thymonucleic acid; both give positive reactions with nuclear chromatin and negative results with virus inclusions.

Nuclear inclusions have also been classified in two types by Cowdry. Type A includes generally bodies in nuclei in which all

of their basophilic chromatin is ultimately marginated; such are found in herpes, yellow fever, virus III disease, pseudorabies and others. In Type B the basophilic chromatin fails to marginate and the acidophilic bodies appear in various parts of the nucleus; they occur in poliomyelitis, Borna's disease, equine encephalomyelitis, and others. Both types may, however, occur in the same disease, as shown by Cowdry,[4] Sabin and Hurst,[17] and by our own observations.

The general characteristics which are commonly ascribed to proved virus inclusions have been mentioned but it is known that variations occur. Most specific intranuclear inclusions are acidophilic but some have been described [17-19] that are either partially or completely basophilic. Presence of halos depends on the particular virus and the stage of infection. In herpes [20] halos appear only in later stages. Margination occurs irregularly; Cowdry, Lucas and Fox [21] have noted it in normal cells and we have observed it in nuclei without inclusions. The histochemical reactions have not been universally applied.

Methods and Materials

Aluminum hydroxide was prepared essentially according to the directions for making Willstätter's Type C gel.[22] This was suspended in $\frac{M}{30}$ phosphate buffer at pH 6.6. Another preparation was secured by adding equal parts of ammonium hydroxide (C. P., 28 per cent NH_3) and a solution of 32 per cent aluminum sulphate with shaking at room temperature. The resulting precipitate was washed with distilled water until free of ammonia, using the centrifuge for sedimentation. A third form of aluminum consisted of alundum, an insoluble form of aluminum oxide commonly used as an abrasive in grinding virus-containing tissues. A weighed amount was thoroughly ground in a mortar with distilled water until a fine suspension resulted. Ferric hydroxide was obtained by adding ammonium hydroxide to a clear solution of ferric chloride and washing in the same way as the Type C gel. Suspensions of barium sulphate, silver chloride and carbon were prepared by adding distilled water to the required amounts. Parowax (a paraffin of moderately low melting point) and 2 per cent nutrient

agar with Witte's peptone were also used. All preparations were autoclaved.

Materials were injected under the skin in guinea pigs and rabbits in amounts of 1 cc. in each site. The object was to produce palpable nodules that could be removed at various intervals of time. It was found that size and persistence of nodules depended mainly on concentration and composition of the material, the depth of injection, and the thickness of the skin. Those of aluminum hydroxide usually lasted 6 to 8 weeks. Ordinarily 7.5 to 10 mg. per cc. of aluminum oxide or of the other chemicals were used but concentrations as high as 20 to 60 mg. per cc. of ferric hydroxide, barium sulphate, or carbon were sometimes necessary to bring about persistent nodules. Paraffin was given undiluted. When the first injection did not result in the formation of nodules, the same sites were reinjected 2 or 3 days later with the same or a higher concentration of the same material. The time of removal of nodules was then counted from the date of second injection.

Nodules were excised * aseptically at various intervals. The skin was closed by clips and small dressings of gauze with collodion were applied. In this way 4 or 5 nodules could be removed from a single animal. The excised tissue was cut so that a piece of skin was retained and then fixed in Zenker's solution containing 5 per cent glacial acetic acid in preparation for ordinary stains to be mentioned, and in special fixatives for certain other stains, as shown in Table II.

Types of Tissue Reactions

During the first few days after subcutaneous inoculation of the mentioned foreign substances in guinea pigs (and in the case of aluminum compounds and of ferric hydroxide, in rabbits), the tissues at the site of injection responded with the usual type of inflammation — chiefly polymorphonuclear cell invasion, edema and congestion. After 4 or 5 days the individual reaction to each of the substances became manifest.

Table I summarizes the types of induced reactions. With the aluminum substances, the hydroxide C gel or ordinary form, or alundum, a foreign body nodule was brought about. The central

* Operations on animals were done with the aid of ether anesthesia.

portion contained heterogeneous material of broken down cells, the injected chemical itself, débris, and a few polymorphonuclear

TABLE I

Substances Used for Injection and Their Effects in Guinea Pigs

Substance	Main features of tissue reaction 1 week after inoculation	Presence of inclusions
Aluminum hydroxide (Willstätter's Type C)	Phagocytic mononuclear cells and giant cells	++*
Ordinary aluminum hydroxide	Same	++
Alundum (Al_2O_3)	Same	++
Ferric hydroxide	Same	+
Barium sulphate	Same	–
Silver chloride	"Cold" abscess surrounded by newly formed connective tissue. No giant cells	–
Paraffin	Strands of fibroblasts surrounding and penetrating the paraffin. Few phagocytic mononuclear and giant cells	–
Carbon	Phagocytic mononuclear cells and giant cells	+
Agar	Same	–

* ++ indicates that numerous inclusions occurred from about the 7th day after inoculation and then persisted throughout the duration of the lesion (for several weeks).
+ indicates that they occurred only temporarily and in small numbers.

and monocytic leukocytes. This was surrounded by a dense mass of phagocytic mononuclear and later of giant cells (Figs. 1 to 6).* A connective tissue band of varying width was formed at the outer

* With certain chemicals (aluminum, iron) the giant cells contained nuclei arranged peripherally; with others (barium, paraffin, and carbon), these cells exhibited nuclei that were scattered throughout the cytoplasm, and with agar both forms, especially the former, were produced. The foreign body was, however, engulfed by the cytoplasm of both types of giant cells. Supravital and Masson's stains were performed only on the aluminum material. Here the precise classification of cells comprising the nodular lesion could be made (with the assistance of Dr. A. L. Joyner) and revealed phagocytic mononuclear, stimulated phagocytic mononuclear, epithelioid, and Langhans' giant cells (those with peripherally arranged nuclei).

margin. The phagocytic mononuclear and giant cells progressively invaded the central material from its periphery and engulfed most of it. The process continued until all miscellaneous contents of the center were removed, ultimately to end in healing by scar tissue formation. The lesions induced by ferric hydroxide were similar. The iron in stained or unstained nodules could be easily identified by its pronounced brown color both in cytoplasm and in the central area (Fig. 7). Barium sulphate after inoculation gave rise to nodular formation, the principal cells in which were phagocytic mononuclear and giant cells. The chemical could be seen both in the cytoplasm of these cells and in the core of the lesion as colorless, ice-like crystals. Silver chloride produced a different kind of lesion — a "cold" abscess of polymorphonuclear cells surrounded by a band of newly formed connective tissue cells, terminating finally in fibrosis. No giant cells were present. With paraffin were found strands of fibroblasts surrounding and penetrating the substance which was not seen in sections since solvents used in preparation had removed it. The lesion also contained a few phagocytic mononuclear and giant cells. Carbon induced localized areas of these cells surrounded by a connective tissue membrane. The black carbon particles were found to be packed into the cytoplasm of both forms of cells. Agar brought about nodules [23] which consisted of these cells with a thin layer of fibroblastic tissue enclosing the lesion. In earlier stages (to 7 days) agar as thin acidophilic strands was noted in the center and as amorphous material in the cytoplasm; later, the agar disappeared except for small, indefinite cytoplasmic masses.

To summarize, there were found in the lesions induced by agar and the chemical substances, except silver chloride, a preponderance of phagocytic mononuclear and giant cells. In those cells in which the cytoplasm visibly engulfed the foreign body, no trace of their presence could be seen in the nucleus (Figs. 3 to 7). The apparent failure to penetrate the nucleus when the cytoplasm was packed to the bursting point by the injected material should be stressed since a problem on the source of any intranuclear inclusions must first take into consideration the possibility of intranuclear entry of particles of the foreign body itself. This negative finding, taken together with others to be described, points to the fact that such invasion did not occur.

Inclusion Bodies

Location and Morphology: Inclusions were present only in the nuclei of the phagocytic mononuclear and giant cells which were produced by aluminum compounds, ferric hydroxide, and carbon, and not in the nuclei in the lesions induced by the other materials. In the aluminum nodules they appeared in small numbers about 7 days after injection, that is, a short time after the phagocytic mononuclear cells were apparent in large numbers. They increased progressively until at about the 24th day there were often 10 to 12 per oil immersion field. Nodules from different animals, however, showed some variation in numbers. How long they persisted depended on the duration of the nodule. Inclusions were first seen with ferric hydroxide on about the 18th day and then only in half the number of animals. At about the 24th day they were not demonstrable. However, when present they were quite numerous. With carbon the acidophilic intranuclear bodies were observed first on the 18th day after inoculation, then again on the 24th day. Thereafter the lesion, as formed by the described method, disappeared. In numbers the bodies were less than in the aluminum material and roughly equaled those found in the iron preparations.

Although the aluminum compounds and ferric hydroxide produced the same reaction in rabbits, yet the inclusions were extremely rare or absent in this animal. This was in contrast with guinea pigs in which they were invariably found after the first 7 days. As a corollary to the presence of inclusions in one species of animal and not in another, reference should be made to the work of Hurst [18] who could not demonstrate intranuclear inclusions in pigs infected with pseudorabies virus, although they were present in other animals, such as the rabbit, guinea pig, monkey and cow.

In stained preparations in the instances in which aluminum was injected, the contrast between the appearance of nuclei containing inclusions and those free from these bodies was striking. Non-inclusion-bearing nuclei were regularly basophilic. Such a nucleus consisted of a round or ovoid nuclear membrane within which were usually one or more nucleoli of various sizes and shapes. In addition, other more finely divided basophilic material was scat-

tered throughout. Minute amounts of acidophilic substance were also rarely seen but were not considered to be inclusions because of their small size, their lack of form, and their occurrence in otherwise normal cells. Amphinucleoli (basophilic nucleoli with acidophilic cores) were not ordinarily present. Basophilic chromatin was occasionally found collected on the nuclear membrane making it appear thicker and comparable to the margination of inclusion-bearing nuclei.

A constant characteristic of the inclusions was their property of acidophilic staining and this was used throughout as the most important criterion for their recognition (Figs. 3 to 7). No structure was thought to be an inclusion that did not stain with phloxine or some other "acid" dye and this made it necessary that differentiation be carried out properly during the staining procedure. Unless the nucleoli of the cells of adjacent tissues were definitely basophilic, the preparation was not considered suitable.

Most of the acidophilic bodies were approximately equal in size to average nucleoli. Others were larger, however, and some nearly filled the nucleus and could be seen without an oil immersion lens (Fig. 2). Great variation was observed with regard to form. Round, flat, whole-edged ones were common; some were angular, others fluffy. A number of them appeared to be composed of minute granules. Ordinarily they appeared less dense than nucleoli and more refractile (Fig. 2). As many as four were found in some nuclei, although one or two was the usual number.

In most instances margination of chromatin occurred, the nucleoli and other basophilic material being found collected peripherally on the nuclear membrane. Clear spaces or halos were usually to be seen around the inclusions. Nuclei containing the bodies at times were slightly larger than their fellows and appeared as a rule as clear sacs in which the ground substance could not be seen.

From their general appearance and also from their morphological characteristics it would seem that the inclusions produced by the substances mentioned are not distinguishable from those that occur in virus-containing tissues. Variations in form occur so that ones corresponding to either of Cowdry's types have been found in a single preparation. From the results of tests to be described immediately, additional similarity can be shown to exist between the chemically induced inclusions and those of virus diseases.

Tinctorial and Histochemical Tests: The purpose of these tests was to determine whether the intranuclear inclusions induced by the action of aluminum and iron compounds were (1) identical in their reaction to those brought about by viruses, (2) merely particles of the introduced foreign bodies, or (3) degenerated nucleoli.

Most of these tests were performed on nodules resulting from injection of aluminum compounds since these showed uniformly formation of abundant characteristic intranuclear inclusions. In Table II will be found a summary of the reactions of the inclusion, the nuclear structures, and the aluminum material engulfed in the cytoplasm.

It will be noted that the inclusions evoked by means of chemicals maintain their acidophilic character with different stains, as is the case with those brought about by viruses. The only exception is iron hematoxylin. With the same stain, however, Sabin and Hurst [17] found the inclusions of B virus to be occasionally basophilic. Moreover, with most of the stains, color differences could be seen between inclusions and the aluminum hydroxide present in the center of the nodule or the cytoplasm of the cell. This suggested that the inclusions were not composed of aluminum hydroxide but did not exclude the possibility that within the nucleus it might have a different color because of combination or adsorption with organic substance there. However, as we have already stated, it was apparent that of all the chemical substances and agar injected, none was found by microscopic examination to have penetrated the nucleus.

The next series of tests concerned the possibility of identifying the inclusions with nuclear material. The Feulgen reaction is a test that has been widely used to detect the presence of thymonucleic acid which is a component of nuclear substance. Cowdry [7, 16] has found that the intranuclear inclusions of certain virus diseases do not give the reaction. It was applied to the inclusions under investigation following his method, except that in addition counterstains of eosin or orange G were used. Like those of virus diseases, these inclusions gave a negative reaction in contrast with the other parts of the nucleus and so presumably did not contain thymonucleic acid.

TABLE II

Staining Reactions of Intranuclear Inclusions in Nodules Produced by Aluminum Hydroxide

Stain, counterstain	Fixative	Inclusions	Nuclear membrane and nucleoli	Aluminum hydroxide in cytoplasm
Methylene blue, phloxine	Zenker's (5% acetic acid)	Red	Dark blue	Lavender or unstained
Methylene blue, eosin	Zenker's (5% acetic acid)	Pink or red	Dark blue	Lavender or unstained
Hematoxylin, eosin	Zenker's (5% acetic acid)	Dark red	Blue-black	Lavender or unstained
Hematoxylin, orange G	Zenker's (5% acetic acid)	Gray	Dark blue	Light yellow or unstained
Giemsa	95% alcohol	Dark red	Dark blue	Gray, lavender, or unstained
Methyl blue, eosin (modified Mann)	Zenker's (5% acetic acid)	Red	Blue	Lavender or unstained
Iron hematoxylin, orange G	Zenker's (5% acetic acid)	Black	Black	Yellow
Masson's connective tissue stain	Helly's fluid	Dull brown or dark gray	Brown	Bright green
Feulgen, eosin	95% alcohol or sublimate alcohol	Red	Purple	Light pink or unstained
Feulgen, orange G*	95% alcohol	Orange	Purple	Light yellow or unstained
Masked iron, eosin	95% alcohol	Dull red	Blue-black	Lavender or unstained
Masked iron, orange G	95% alcohol	Yellowish gray	Blue-black	Light yellow or unstained

* This counterstain was used as recommended by J. A. de Tomasi (*Stain Technology*, 1936, 11, 137).

Cowdry [16] has also found that the virus inclusions he studied reacted negatively to the Macallum test for masked iron. This is a test for iron in organic combination such as is present in the basophilic chromatin of normal nuclear material. The procedure was carried out essentially according to the directions of Nicholson [24] and Lee.[25] Sections were first allowed to stand in a solution of 4 per cent sulphuric acid in order to "unmask" the iron, that is, change it into inorganic form. This was followed by a yellow solution of pure crystalline hematoxylin * which combined with this iron and gave a blue color. A counterstain of eosin or orange G was then applied. Again like those of virus diseases, these inclusions gave a negative reaction for masked iron.

Preliminary work with Zenker-fixed tissue on the application of the test for masked iron to nodules of ferric hydroxide showed that this substance itself did not react but retained a brownish yellow color similar to that in sections stained by ordinary methods. It was therefore considered unlikely that this method could give conclusive evidence as to whether ferric hydroxide had appeared within the nucleus modified in such a way as to stain as inclusions. However, such a possibility seemed remote in view of the fact that this iron compound showed such a distinctive color and apparently nowhere penetrated the nucleus.

The method of microincineration has also been applied to determine the mineral content of several virus inclusions.[20, 26-28] The procedure was employed in the present study essentially as described by Scott.[28] With the material studied, the results were disappointing since the nodules contained quantities of inorganic substance which left an abundant ash after the organic material was burned away. The ash left by the aluminum hydroxide covered the whole area in which inclusion-bearing nuclei were to be sought, thus obscuring the fields under observation.

Other Tests: Sodium alizarin monosulphonate added to salts of aluminum gives a red color; this reaction offers a delicate test for this element. Attempts were made to develop a histochemical test using this dye with the idea of detecting the presence of

* When the preliminary treatment with acid was omitted from control sections the nuclei were not colored with this solution. 95 per cent alcohol was used for fixation because when such a control was used with sections of tissue fixed in Zenker's solution the nuclei gave a non-specific reaction.

aluminum in the inclusions. All such trials have ended in failure because the aluminum hydroxide in the center of the nodule or in the cytoplasm would itself never give a positive test. It is a very insoluble substance and any procedure designed to bring it into solution has a deleterious effect on the organic structures in the section. That aluminum was still present at the times nodules were removed was shown by gross chemical tests.

The method of supravital staining was tried on inclusion-bearing nodules. One could see the nucleolus eccentrically placed in the nucleus and suggestions of margination of nuclear material. Moreover, in some of such nuclei, bodies lighter in texture, more homogeneous, and resembling inclusions were seen. However, it could not be said with certainty that these were the same structures that were seen in fixed preparations.

From the results of these tests it would seem that the intranuclear inclusions evoked by the injection subcutaneously in guinea pigs of the aluminum and iron compounds have many resemblances to virus inclusions. The similarity exists not only in morphology and location but in the response of the bodies to the Feulgen and masked iron tests. Furthermore, the recorded reactions point to the fact that the inclusions under investigation are not merely degenerated nucleoli or artefacts due to technical procedures, and finally that they do not in themselves represent particles of injected chemicals.

Search for Virus

The presence of inclusions suggested the action of a virus (1) preexisting in the animals and activated by the injected materials; (2) "formed *de novo*" as questioned by Cowdry [3, 7] as a possible occurrence if intranuclear inclusions were produced by artificial means; (3) being a contaminant, perhaps one of the viruses studied in this laboratory, and (4) carried along with the materials used. The fourth can be eliminated by the fact that such substances were autoclaved. Because of the other three possibilities, evidence of a virus was sought. An outstanding example of where the finding of intranuclear inclusions led to the discovery of a virus is that afforded by the work of Cole and Kuttner [29] on the salivary gland virus of guinea pigs. This infective agent is ap-

parently present in most of the guinea pigs of certain stocks without giving rise to symptoms but is, nevertheless, associated with intranuclear inclusions. Such inclusion-containing material is, however, capable of transmission in series to young guinea pigs which then show the clinical disease with death.

Transmission Experiments: The purpose was to select animals believed to be most susceptible and to inoculate into them by various routes heavy suspensions of inclusion-containing tissue. Nodules resulting from injection of aluminum compounds were used as source material. As we have already mentioned, these compounds formed numerous inclusions regularly.

Nodules were removed at intervals of 3, 7, 10, 12, 17 and 24 days. These were ground aseptically with alundum and enough sterile Tyrode's solution to make dilutions from 1:5 to 1:10. After light centrifugation to deposit gross particles including the alundum, the supernatant liquid was used for inoculation. All the injected animals and the original ones bearing nodules were kept in a separate room and isolation precautions were taken When the nodules were removed, part was taken for section so that it could be determined whether the material actually used for passage contained inclusions. All of these nodules, except the one removed at 3 days, did show inclusions.

A total of 36 mice was given intracerebral injections of 0.03 cc. each of such material. One died of the inoculation; the rest survived for 1 to 2 weeks and during this time showed no clinical evidence of disease. The brains of 3 of these mice were removed for section after 7 to 12 days of observation but no lesions attributable to the action of a virus were found and no inclusions seen.

Fifteen young guinea pigs (10 to 12 days old) were given intracerebral inoculations of 0.05 cc. each. In addition, 3 of them received subcutaneous doses of 1 cc. One died of a perforated colon, and the brains of 4 others were removed for section on the 12th to 15th days. No lesions or inclusions were found. The remaining ones were observed for 2 to 3 weeks but in none did clinical signs of disease develop.

Twelve adult guinea pigs were each given intracerebral inoculations in doses of 0.1 cc., subcutaneous inoculations in doses of 1 cc., and intradermal and subcutaneous inoculations in a pad. The brains of 4 of these were removed for section at intervals of 2 to

12 days. The pads of 6 were removed for section at intervals of 2 to 12 days. Neither brains nor pads showed lesions or inclusions. Barely perceptible transitory indurations appeared in 6 of the animals at the sites of subcutaneous injection. No reactions whatever occurred in others in which the inoculum had been centrifuged more strongly. Skin and subcutaneous tissue at the site of injection were removed for section at intervals of 2 to 12 days from 6 animals. Microscopically the indurations consisted of minute collections of phagocytic mononuclear cells. In 2 of the sections occasional inclusions were found. In these cases tissue had been removed 12 days after subcutaneous inoculation. It was believed that in the instances in which minute indurations were produced these areas contained sufficient chemical which was carried along with the inoculum and set up a slight transient reaction. None of the adult guinea pigs showed clinical signs of infection.

Tissues of animals that had been injected with ground nodule in attempts at transmission of virus were, in turn, ground again and injected into other guinea pigs, thus effecting a second "passage." In 1 test, 2 of the minute, subcutaneous indurations described above were ground with alundum and Tyrode's solution. The supernatant fluid after centrifugation was injected subcutaneously into 6 adult guinea pigs in doses of 1 cc. Four of the 6 developed almost imperceptible indurations that disappeared in about 1 week and the sites of 2 of these were removed at 12 and 13 days for section but no lesion other than some increase in fibroblastic tissue was seen. In another test, brains of 4 young guinea pigs which had received intracerebral injections were ground with alundum and sterile Tyrode's solution. After centrifugation they were injected intracerebrally into 6 adult guinea pigs in doses of 0.1 cc. No signs of infection resulted.

It is therefore apparent from the tests performed on mice and guinea pigs that were inoculated with nodular tissues containing inclusions that no evidence of virus infection was obtained.

Reinoculation Test: One might assume that the association of a possible virus with the nodules might set up an immunity so that subsequent injections of aluminum hydroxide would not produce inclusions. An experiment was planned to test this point.

Five guinea pigs were each given 3 inoculations of aluminum hydroxide. One nodule was removed for section from each in 12

to 30 days; these were found to contain the characteristic intranuclear inclusions. On the 41st to 56th day after first injection these animals and a normal control were reinoculated. Twelve or 24 days later nodules derived from the reinjection were removed for section. In addition, some of the first nodules, now 53 days old, were examined.

Inclusions were found in all of the sections of old and new nodules. Consequently no immunity, as postulated, could be shown to exist, since typical new intranuclear structures could be produced, regardless of whether others were present at the time or had previously been demonstrated in the same animals.

Examination of Submaxillary Glands: In view of the fact that the inclusions were observed in guinea pigs but not in rabbits and that adult guinea pigs are known to be infected often spontaneously with salivary gland virus, an attempt was made to obtain evidence of its presence. In addition to the transmission experiments already described, this was done by examining for virus inclusions the submaxillary glands of guinea pigs in which nodules produced by aluminum hydroxide had shown inclusions.

Right and left submaxillary glands were removed for section from 4 adult guinea pigs in which the nodules formed by means of aluminum hydroxide had shown many inclusions. The tissues of 2 of these animals revealed occasional intranuclear inclusions typical of the salivary gland virus. The remaining 2 showed none. Two young (10 day old) guinea pigs were given injections of aluminium hydroxide. Nodules were removed on the 7th and 18th days afterwards and also on the latter date (28th day of life) the right and left submaxillary glands were excised for study. Inclusions were found in all 4 of the nodules but in none of the submaxillary glands.

Reference is also made to the fact that in the transmission experiments to young guinea pigs with nodular material, no infection with salivary gland virus was discernible in inoculated animals. Moreover, the characteristic basophilic marginal structures surrounding the intranuclear acidophilic inclusion body of salivary gland disease was not generally seen in the nodules induced by aluminum compounds. It is therefore not likely that the intranuclear inclusions found in the latter represent the bodies brought about by this virus.

In the foregoing experiments attempts were made to detect the presence of any virus that might possibly have been the causal factor in the production of the described intranuclear inclusion bodies. These trials were made by means of animal inoculation of affected tissues, by tests to show whether immunity was induced, and by histopathological studies. What were believed to be the most favorable routes of inoculation and the most susceptible animals were employed. From the results it is apparent that a virus was not demonstrated under these experimental conditions.

Discussion and Summary

In the present paper is shown the formation of intranuclear inclusion bodies by the subcutaneous injection of guinea pigs with different aluminum compounds, less often with ferric hydroxide and carbon. They appear to resemble virus inclusions so closely as to be practically indistinguishable from them. One might add that in the instances in which ferric hydroxide and carbon were used they appeared after a prolonged interval and then only for a short period during the height of the reaction. Similar occurrences are known in virus pathology. With aluminum compounds, after the initial period of about 7 days passed, they were observed throughout the duration of the lesion. Finally, special types of cells, phagocytic mononuclear and giant cells, only are involved, and not all foreign body tissue reactions contain the inclusions — barium sulphate, silver chloride, agar, and paraffin have failed.*

The mechanism underlying the production of these chemically induced inclusions is unknown. Just as with virus inclusions they fail to give the Feulgen (thymonucleic acid) and the masked iron reactions; this is taken as evidence that they do not originate from degenerated nucleoli. Another source might be particles of the introduced foreign substances. In this connection Jacobs,[30] in an article on cell permeability, warns against assuming that a colored substance has not penetrated a cell merely because it cannot be seen. While nuclei are being dealt with and not cells as a whole, a

* No inference is intended that the aluminum and iron compounds and carbon are the only substances that can possibly give rise to inclusions.

conclusion cannot be reached on this basis as to whether or not the inclusions within the nuclei are particles of the injected substances. This possibility is remote, however, in view of the visible material used, and since in no instance could any part of it be observed to have entered the nucleus.

The point to be stressed is that the intranuclear inclusions produced by chemical means are apparently not associated with a virus; a search which included a number of transmission experiments to animals of the same species in which they were originally produced gave a negative result. In view of this, it is evident that no disease can be classified as a virus disease on evidence of finding in pathological examination of tissues intranuclear inclusion bodies unless it can be proved that they are associated with a virus, that is, unless experimental transmission can be effected.*

While intracytoplasmic inclusions occurring in the lesions set up by viruses are generally considered as colonies of virus particles — they are commonly found associated with such infective agents of larger size — it becomes more difficult to interpret the significance of virus intranuclear inclusions. One interpretation offered is that changes called forth in the cytoplasm due to virus action might possibly induce changes in the nucleus with the accompanying formation of the intranuclear bodies. From what is here reported, it would appear that a similar sequence of events might follow the engulfing of foreign bodies by cytoplasm, which in turn might influence the nucleus in the manner already described. It is, however, unknown whether or not the structures within the nucleus that appear to resemble so closely those associated with viruses are identical with them in their physicochemical natures.

Conclusions

1. Intranuclear inclusion bodies resembling in certain important ways those found in virus diseases were induced by chemical substances such as selected aluminum and ferric compounds and

* Alundum is used commonly as an aid in grinding virus tissues. It has been shown that alundum itself may induce localized tissue reactions associated with virus-like inclusion bodies. It is therefore conceivable that such inclusions might be mistaken for bodies induced by viruses if care is not taken to centrifuge the alundum out of tissue suspensions to be injected.

carbon but not by others such as barium sulphate, silver chloride, and paraffin, nor by agar.*

2. No evidence was revealed of a virus infection in association with these inclusions.

3. The results throw light on the interpretation of intranuclear inclusions as they occur in lesions caused by viruses.

REFERENCES

1. Cox, Herald R., and Olitsky, Peter K. Prevention of experimental equine encephalomyelitis in guinea pigs by means of virus adsorbed on aluminum hydroxide. *Science*, 1934, **79**, 459.

2. Cox, H. R., and Olitsky, P. K. Unpublished experiments.

3. Cowdry, Edmund V. Intracellular pathology in virus diseases. Filterable Viruses, Rivers, Thomas M. Williams and Wilkins, Baltimore, Md., 1928, Chap. 4, 113–154.

4. Cowdry, E. V. The problem of intranuclear inclusions in virus diseases. *Arch. Path.*, 1934, **18**, 527–542.

5. VonGlahn, William C., and Pappenheimer, Alwin M. Intranuclear inclusions in visceral disease. *Am. J. Path.*, 1925, **1**, 445–466.

6. Farber, Sidney, and Wolbach, S. Burt. Intranuclear and cytoplasmic inclusions ("protozoan-like bodies") in the salivary glands and other organs of infants. *Am. J. Path.*, 1932, **8**, 123–136.

7. Cowdry, E. V. A comparison of the intranuclear inclusions produced by the herpetic virus and by virus III in rabbits. *Arch. Path.*, 1930, **10**, 23–37.

8. Blackman, S. S., Jr. Intranuclear inclusion bodies in the kidney and liver caused by lead poisoning. *Bull. Johns Hopkins Hosp.*, 1936, **58**, 384–403.

9. Lee, Jack. Nuclear alterations following intravenous injections of glucose and of other solutions. *Am. J. Path.*, 1936, **12**, 217–234.

10. Seifried, O. Vergleichende Histo- und Cyto-Pathologie der Virus-Infektionskrankheiten. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1936, **31**, 201–235.

11. Cowdry, E. V., and Kitchen, S. F. Intranuclear inclusions in yellow fever. *Am. J. Hyg.*, 1930, **11**, 227–299.

12. Olitsky, Peter K., and Long, Perrin H. Histopathology of experimental vesicular stomatitis of the guinea pig. *Proc. Soc. Exper. Biol. & Med.*, 1928, **25**, 287–288.

* After this paper was submitted for publication, intranuclear inclusion bodies similar to those described here were seen in subcutaneous nodules induced in guinea pigs by injection of brain tissue derived from apparently normal animals. This aspect of the problem is now being studied.

13. Akiyama, Seiroku. Über den Einfluss des elektrischen Stromes und die Wirkung verschiedener Ionen auf die Nervenzellen, insbesondere auf ihre Nisslschen Schollen. *Arb. a. d. Mediz. Univ. z. Okayama,* 1929, **1**, 278–298.

14. Heinbecker, Peter, and O'Leary, James L. A method for the correlation of cell types with fiber types in the autonomic and somatic nervous systems. Abstr., *Anat. Rec.,* 1930, **45**, 219.

15. Lee, Jack. Nuclear changes following intravenous injection of various solutions. *Proc. Soc. Exper. Biol. & Med.,* 1933, **31**, 383–385.

16. Cowdry, E. V. The microchemistry of nuclear inclusions in virus diseases. *Science,* 1928, **68**, 40–41.

17. Sabin, Albert, and Hurst, E. Weston. Studies on the B virus. IV. Histopathology of the experimental disease in *rhesus* monkeys and rabbits. *Brit. J. Exper. Path.,* 1935, **16**, 133–148.

18. Hurst, E. Weston. Studies on pseudorabies (infectious bulbar paralysis, mad itch). I. Histology of the disease with a note on the symptomatology. *J. Exper. Med.,* 1933, **58**, 415–433.

19. Nicolau, S., Kopciowska, L., and Mathis, M. Étude sur les inclusions de la fièvre jaune. *Ann. Inst. Pasteur,* 1934, **53**, 455–508.

20. Rector, L. E., and Rector, Eleanor J. The microincineration of herpetic intranuclear inclusions. *Am. J. Path.,* 1933, **9**, 587–592.

21. Cowdry, E. V., Lucas, Alfred M., and Fox, Herbert. Distribution of nuclear inclusions in wild animals. *Am. J. Path.,* 1935, **11**, 237–252.

22. Willstätter, Richard, and Kraut, Heinrich. Zur Kenntnis der Tonerdehydrate. *Ber. Deutsche chem. Gesellsch.,* 1923, **56**, 149–162.

23. Doan, Charles A., Sabin, Florence R., and Forkner, Claude E. The derivation of giant cells with especial reference to those of tuberculosis. *J. Exper. Med.,* 1930, **52**, Suppl. No. 3, 89–111.

24. Nicholson, F. M. The changes in amount and distribution of the iron-containing proteins of nerve cells following injury to their axones. *J. Comp. Neurol.,* 1923, **36**, 37–87.

25. Lee, Arthur Bolles. The Microtomist's Vade-Mecum. P. Blakiston's Son & Co., Philadelphia, 1924, Ed. 8, 297–299.

26. Cowdry, E. V. The microincineration of intranuclear inclusions in yellow fever. *Am. J. Path.,* 1933, **9**, 149–164.

27. Horning, E. S., and Findlay, G. M. II. Microincineration studies of the liver in Rift Valley fever. *J. Roy. Micr. Soc.,* 1934, **54**, 9.

28. Scott, G. H. A critical study and review of the method of microincineration. *Protoplasma,* 1933, **20**, 133–151.

29. Cole, Rufus, and Kuttner, Ann G. A filterable virus present in the submaxillary glands of guinea pigs. *J. Exper. Med.,* 1926, **44**, 855–873.

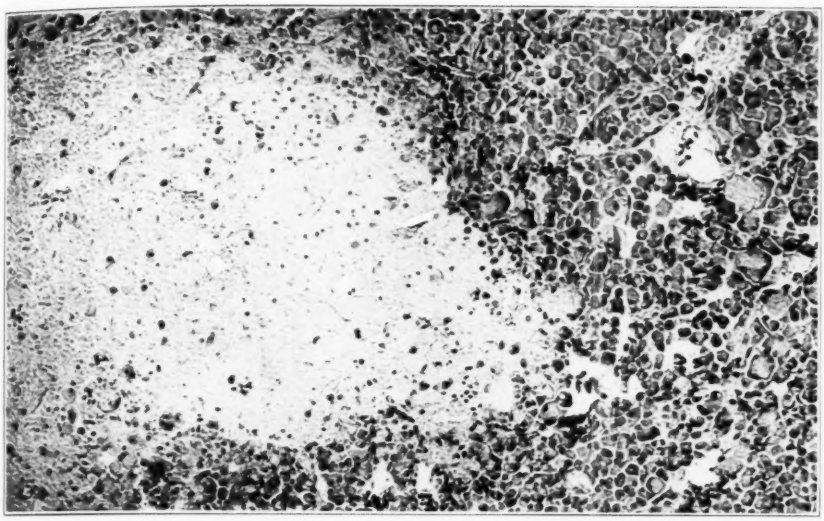
30. Jacobs, M. H. Permeability of the cell to diffusing substances. General Cytology, Cowdry, Edmund V. University of Chicago Press, Chicago, Ill., 1924, Section 3, 97–164.

DESCRIPTION OF PLATES

PLATE 102

FIG. 1. Section of a nodule produced by aluminum hydroxide removed from a guinea pig 24 days after injection. The tissue reaction of phagocytic mononuclear and giant cells is shown about the central portion of tissue detritus and chemical. Phloxine-methylene blue. × 100.

FIG. 2. Same section as Fig. 1. Several epithelioid giant cells with peripherally arranged nuclei are present in this field. Arrows point to inclusions. Note that these bodies appear less dense than the nucleoli in their own and adjacent nuclei. Uppermost arrow indicates a large, herpes-like inclusion. Phloxine-methylene blue. × 1000.



1

2

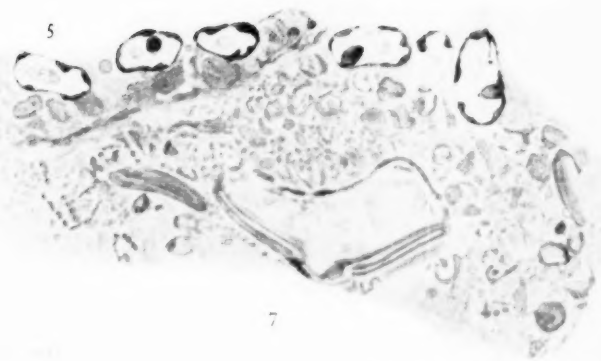
UNIV OF MICH.

3

4

6

5



7

UNIV. OF MICH.

VIABLE PNEUMOCOCCI AND PNEUMOCOCCIC SPECIFIC SOLUBLE SUBSTANCE IN THE LUNGS FROM CASES OF LOBAR PNEUMONIA *

ROBERT N. NYE, M.D., AND ALBERT H. HARRIS, 2ND., M.D.

(From the Mallory Institute of Pathology, Boston City Hospital, Boston, Mass.)

Although the qualitative bacteriology of the lungs in lobar pneumonia is well known, and although some attempts have been made to estimate the number of bacteria by the examination of stained sections, quantitative methods have apparently never been applied to a determination of the number of viable bacteria. Furthermore, although it has been recognized that the consolidated lobes contain large amounts of specific soluble substance, quantitative estimations have not been made.

It was felt that such determinations might furnish information that would be valuable in the study of pneumonic processes during various stages of the disease. The following experiments were conducted with this idea in mind.

MATERIAL AND METHODS

All material was obtained from routine cases coming to autopsy at the Mallory Institute of Pathology. In each instance the lungs and heart were removed en masse. An area of each lobe was seared and a window was cut away from the sterilized area. A block of tissue weighing from 3 to 10 gm. was removed with sterile instruments to a tared Petri dish. A slice of tissue about 3 mm. in thickness was placed in Zenker's solution. In most instances smears and surface cultures on sheep's blood agar plates were made from the exudate that welled up in the cavity. The pulmonary vessels and bronchi were severed at the hilus of each lung, and the lobes were separated one from another. Each lobe was weighed individually.

Each Petri dish was weighed and the net weight of the block

* This work was aided in part by a grant from the William W. Wellington Fund, Harvard Medical School, Boston, Mass.

Received for publication May 7, 1937.

of tissue was calculated. With precautions against contaminating the tissue, it was then transferred to a mortar, in which it was thoroughly minced with scissors and then ground for from 4 to 5 minutes with sterile sand. The mixture was washed into a large test tube (200 by 25 mm.) with an amount of Locke's solution equal to five times the weight of the block. After thorough mixing, 1 cc. of the fluid portion was transferred to a tube containing 9 cc. of beef infusion broth, and serial dilutions were made in the same medium; the number of dilution tubes used in each series was governed by the number of bacteria seen in the Gram-stained smear of the original exudate. Pour plates with sheep's blood agar were made, each containing 1 cc. of the desired broth dilutions. Furthermore, 1 or 2 drops of sterile sheep's blood were added to each broth dilution tube. The entire procedure consumed from 3 to 4 hours. Ten drops of chloroform were added to the mixture in each of the large test tubes, which were then closed with rubber stoppers and slowly rotated in a cold room (2–4°C.) for a week or more.

All cultures were incubated at 37°C. and were examined at the end of 24 and 48 hours. The colonies in the pour plates containing moderate numbers were identified and counted. When colonies were typically those of pneumococci, only 1 or 2 were identified by agglutination tests, whereas, when the identity of the colonies was questionable, the organisms from 10 to 20 colonies were tested for bile solubility, inulin fermentation and agglutinability. The numbers of bacteria per milligram of lung tissue were calculated, assigning the value of 1.0 to the specific gravity of the tissue, which value was considered sufficiently accurate for the purposes of calculation. The numbers of bacteria were designated ±, +, ++, or +++, depending on whether there were less than 10^2, 10^2 to 10^3, 10^3 to 10^5 or greater than 10^5 bacteria per milligram, respectively. The broth dilution tubes served as checks on the pour plates, and in several instances where the dilutions used for pour plates were too high, because there were a great deal fewer viable organisms present than had been anticipated, the highest dilution tube revealing growth of pneumococci was arbitrarily considered to have contained one diplococcus. The surface blood agar plates of the exudate furnished rough checks on the numbers and kinds of bacteria.

Samples of from 1 to 2 cc. were removed from the mash tubes in the cold room every 24 or 48 hours until the maximum content of specific soluble substance (SSS) had developed. This usually required a period of from 4 to 7 days. The samples were centrifuged at high speed (12,000 RPM) for about 30 minutes,* and the clear supernatant fluid, serially diluted in isotonic salt solution, was overlaid on undiluted homologous and heterologous antipneumococcic serums contained in tubes with an approximate inside diameter of 3 mm. The highest dilution giving a faint ring with the homologous serum was noted, and the actual amount of SSS in that dilution was calculated, using as a standard the highest dilution of homologous purified SSS ** producing a similar ring when overlaid on a portion of the same antiserum. In making serial dilutions one pipette was used for each series. The total amount of SSS in each lobe was calculated, based on the assumption that the precipitinogen taking part in the reaction was entirely SSS and that the tissue examined represented a fair sample of the whole lobe. These rough measurements and rather loose assumptions are considered valid only in demonstrating the very wide differences that were found to exist in the amount of SSS contained in the lobes at various stages of consolidation. In 2 cases the SSS was determined in the liver, spleen, blood serum, urine and pleural fluid. For the liver and spleen the method was identical with that for the lungs. For the blood serum, urine and pleural fluid, simple dilutions in isotonic salt solution were employed.

The Zenker-fixed tissue from the lungs was embedded in paraffin and sections were cut approximately 4 μ in thickness. One set of sections was stained with phloxine and methylene blue and another with Rudinkoff and Stawsky's modification [1] of Brown and Brenn's method for differential staining for Gram-positive and negative bacteria. With the optical system used — Zeiss 90 objective and 10 x ocular — each field was equal to approximately 1/14,000 of a cubic millimeter. The sections were carefully

* This was done in a No. 1 centrifuge (International Equipment Co.) with a high speed attachment. The head was always chilled in a cold room before use to prevent overheating.

** These were kindly supplied by Dr. John Enders, Department of Bacteriology, Harvard Medical School, and Dr. Maxwell Finland, Thorndike Memorial Laboratory, Boston City Hospital.

TABLE I — *Histological, Bacteriological*

Clinical, bacteriological and statistical data		Lobe	Gross pathology	Histological			
				Pneumococci			
				Alveoli		Bronchi	
				Free	Phag.	Free	Phag.
Case 10 W ♂ 43 Lobar pneumonia Type I — 5 days duration Autopsy — 3 hrs. PM	Sp (3) Pk I, BMC VB (A) Pk I Pl F (A) L (400 cc.) Pk I L (A) BC S (A) BMC Antiserum — 60 cc. (3, 4)	**RUL** RML RLL **LUL** LLL	Mod. late consol. Sl. edema Sl. edema Late consol. Sl. edema (RBC)	o + +++ + +	+ ++ ++ o o	o o +++ o o	+++ +++ +++ o o
Case 4 W ♂ 39 Lobar pneumonia (Cardiac disease) Type I — 6 days duration Autopsy — 17 hrs. PM	Sp (6) Pk I VB (A) Pk I Antiserum — 5 cc. (6)	**RUL** RML RLL LUL LLL	Mod. late consol. Normal Mod. late consol. Normal Mod. late consol.	o o o + +	o o o + o	o o o o ++	o +++ o o ++
Case 2 W ♂ 37 Lobar pneumonia (Alcoholism) Type I — 8 days duration Autopsy — 4 hrs. PM	Sp (4) Pk I Pl F (A) R (200 cc.) o	**RUL** RML RLL LUL LLL	Late consol. Mod. late consol. Mod. late consol. Normal Normal	o o o o o	o o ++ o o	o o o o o	o o o o +
Case 11 W ♂ 74 Lobar pneumonia (Alcoholism) Type I — 8 days duration Autopsy — 5 hrs. PM	Sp (2) Pk I VB (A) Pk I L (A) BW S (A) BW, BC	RUL RML RLL **LUL** LLL	Congestion (RBC) Congestion (RBC) Congestion (RBC) Mod. late consol. Mod. late consol.	o .. o + +	o .. o ++ +++	o .. +++ ++ ++	++ .. + ++ ++
Case 3 W ♀ 70 Lobar pneumonia (Cardiac disease) Type I — 12 days duration Autopsy — 10 hrs. PM	VB (8) Pk I	RUL RML **RLL** LUL LLL	Normal Absent Late consol. Normal Early consol. (RBC)	o .. o o o	+++ .. ++ ++ +++	o .. o ++ +++	+++ .. +++ +++ +++
Case 1W ♂ 18 Lobar pneumonia Type II — 13 days duration Autopsy — 3 hrs. PM	Sp (4) Pk II VB (4, 9, 11) Pk II Pl F (A) R (500 cc.) o L (700 cc.) o Antiserum — 225 cc. (4, 5)	RUL RML **RLL** LUL LLL	Lymphangitis Late consol. (lymphangitis) Late consol. Normal Mod. late consol. { lym'tis / RBC }	o o ++ o o	o o +++ o +++	o o o o o	++ o +++ o o
Case 6 W ♀ 72 Lobar pneumonia Type III — 8 days duration Autopsy — 11 hrs. PM	Sp (7) Pk III VB (8) Pk III	**RUL** RML RLL LUL LLL	Mod. late consol. Early consol. Early consol. Congestion (RBC) Late consol.	o .. ++ ++ ++	+++ .. +++ o +++	o .. o ++ +++	+++ .. o o +++
Case 8 W ♀ 50 Lobar pneumonia Type III — 8 days duration Autopsy — 4 hrs. PM	Sp (6) Pk III VB (7) Pk III Pl F (A) R (500 cc.) o	RUL **RML** RLL LUL LLL	Normal Mod. late consol. Early consol. Normal Sl. edema	o o o o o	o o +++ o o	o o o +++ ++	o o +++ ++ ++
Case 9 W ♂ 51 Lobar pneumonia Type III — 8 days duration Autopsy — 12 hrs. PM		RUL RML **RLL** LUL LLL	Early consol. Late consol. Resolving consol. Congestion and edema Congestion and edema	o o +++ o +++	+++ + o o o	o o + o o	++ o + o o

General Data: Sp = sputum, VB = venous blood, Pl F = pleural fluid (R = right, L = left), L = liver, S = spleen, RBC = red blood corpuscles, Pk = *Pneumococcus*, BMC = *B. mucosus capsulatus*, BW = *B. Welchii*, and BC = *B. coli*. Numbers in parentheses represent day of disease; A = autopsy.

Lobe: Bold face type represents the primarily involved lobe.

bacteriology	Cultural bacteriology		pH	Soluble specific substance		Appreciable numbers of viable pneumococci			
Other bacteria	Pneumococci	Other bacteria		Lungs	Other organs	Norm. lobes	Cong. or ed. lobes	Cons. lobes	Bronchi
				mg.	*mg.*				
	o	BMC ±	..	800	Pl F 200			o	
	++	BMC +++	..	20	B 40		+		
BMC +++ FGPB ++	++	BMC +++ SV ++	..	400	U 4		+		
GNB ++	++R	BMC +++ SV ++	..	1000	L & S < 10			+	
BMC ++	o	BMC +++	..	60	Total – 2500		o		
	±		5.7C	100	B 4			o	
	±	SA ±	6.0	1	U 1	o			
	o	SA ± SV ±	5.5	400	L & S 11			o	
GPB ++ GPC ++	+	SA ++ SV +	6.3	3		o			+
	o	SA ± SV ±	6.0	100	Total – 600			o	
	±		7.2C	20				o	
	±		7.0	80				o	
	±		6.5	400				o	
	±		7.6	1		o			
	±		7.4	1	Total – 500	o			
	++	BMC ±	6.9E	↓			o		+
	..		..	20					
	++	BMC ±	7.4	↑			o		+
	±		6.9	1600				o	
	±	BMC ± SA ±	7.1	200	Total – 1800			o	
FGPB ++	+	Pk (XXX) +	7.0C	1		o			+
	..		..	..					
FGPB ++	o		6.2	23				o	
	++		7.0	1		o			+
GPC ++ GNB ++	+		6.9	7	Total – 30			o	+
	±		7.4C	9		o			
	±		6.3	20				o	
	±		7.5	600				o	
	±		7.4	1		o			
	±		6.1	600	Total – 1200			o	
GPC ++	o		..	5800				o	
	±	SA ++	..	200				o	
	±	SA ++	..	500				o	
GPC +++	+++	SA +++	..	80			+		
GPC +++ GPB ++ GNB ++	±	SA ±	..	1800	Total – 8400			o	
	±		..	?		o			
	±		..	200				o	
	±	SV ±	..	300				o	
GNC +++	++	SV +	..	500		o			+
GNC +++ GPC +++	++	SV ++	..	700	Total – 1700		o		+
GPB ++ GNB ++	±		6.0E	600				o	
	++		6.1	200				+	
FGPB +++ GPC +++ GNC +++	++	SV +++ SA ++ SH ++	6.7	20				+	
FGPB +++	++	SV +++ SA ++	7.2	5			+		
FGPB +++ GPC +++ HI +++	+++	SV +++ SA +++	7.0	20	Total – 850		+		

Histological Bacteriology: BMC = bacilli similar to *B. mucosus capsulatus*, FGPB = fat Gram-positive bacilli, GNB = Gram-negative bacilli, GNC = Gram-negative cocci, GPC = Gram-positive cocci, and HI = bacilli similar to *H. influenzae*.
Cultural Bacteriology: BMC = *B. mucosus capsulatus*, SV = *Streptococcus viridans*, SA = *Staphylococcus aureus*, Pk = *Pneumococcus*, SH = *Streptococcus hemolyticus* and R = rough.
pH: C = colorimetric and E = electrometric.
SSS: B = Blood, U = Urine.

studied. The predominant type of consolidation in each lobe was observed and noted. Those designated "early" showed an exudate in the alveoli that consisted, chiefly, of neutrophilic polymorphonuclear leukocytes embedded in a meshwork of fine fibrin strands; thromboses in the small blood vessels of the alveolar walls were frequently noted. In those considered "late," the exudate contained few to many mononuclear leukocytes; the polymorphonuclear leukocytes as a rule showed degenerative changes, the fibrin plugs were shrunken and partly digested, and the alveolar walls were thickened because of dilatation of the capillaries and proliferation of cells lining the alveoli.

The numbers of free and phagocytosed pneumococci and other bacteria in the alveoli and in the bronchi were designated +, ++ or +++ depending on whether there were a few in many fields, a few in almost all fields or moderate numbers to many in almost all or all fields, respectively.

These designations were roughly comparable to those used for the cultural results. For example, 1 pneumococcus in 14 fields would be called a "+" histologically and, taking into account the volume of one field (1/14,000 cmm.), this would mean 1000 pneumococci per cubic millimeter which, as has been previously noted, would be called a "+" culturally.

The hydrogen ion concentrations were determined colorimetrically or electrometrically, using small amounts of relatively fresh lung suspensions.

Results

All of the findings in each case are given in Table I, together with a certain amount of clinical and pathological data.

Pneumococci: There is fairly good agreement between the numbers of free pneumococci observed in the sections and those recovered culturally. It should be realized that many of the former may not have been alive at the time the sections were taken and counts of viable organisms were made and that many of the latter may have come from the exudate in the bronchi or bronchioles. Provided, however, due allowance is made for contamination from this extraparenchymal exudate, based on a careful study of the stained section, the numbers recovered culturally probably

represented fairly accurately the viable pneumococci in the lung substance at the time of the autopsy.

As shown in Table II, appreciable numbers of viable pneumococci were recovered from only 3, 13 per cent, of the consolidated lobes, namely, from 1 lobe in Case 10, from which only rough variants were cultured, and from 2 lobes in Case 9, in which the consolidation was very late. In the latter case, the presence of very large numbers of pneumococci in the congested and edematous lobes suggests the possibility that the consolidated lobes may have become reinfected shortly before death. The normal lobes contained no viable pneumococci that could not be justly accounted

TABLE II

Summary of Incidence of Viable Pneumococci in All Lobes

Condition of lobe	None to few pneumococci	Moderate numbers to many pneumococci	Probable bronchial contamination	Corrected incidence of appreciable numbers of pneumococci		
				Lobes		Per cent
				Negative	Positive	Positive
Normal	6	4	4	10	0	0
Congestion or edema or both	1	8	3	4	5	56
Consolidation	20	4	1	21(24*)	3(0*)	13(0*)

* If exceptions, noted in the text, are taken.

for by contamination from bronchial or bronchiolar exudate, whereas they were found in 56 per cent of the lobes with congestion or edema or both. It should be noted that extravasated serum and a certain number of red blood cells were always found in the alveoli of lobes revealing marked congestion.

Variation in the interval between death and autopsy had no evident effect on the above findings.

Other Bacteria: Bacteria, other than pneumococci, were seen in the sections or recovered in culture from all but 2 of the 9 cases. The incidence is shown in Table III.

The 2 cases (No. 9 and No. 10) that had the greatest amount of edema in the non-consolidated lobes showed by far the greatest number of bacteria other than pneumococci.

Phagocytosis: Phagocytosed bacteria were seen in nearly all of the preparations, most commonly in the exudate in the bronchi

TABLE III

Summary of Incidence of Bacteria Other than Pneumococci in All Cases

Sections		Cultures	
Description of bacteria	Number of cases	Organisms	Number of Cases
Gram-positive cocci	5	*Streptococcus viridans*	4
Gram-negative bacilli, slender	4	*Staphylococcus aureus*	4
Gram-positive bacilli, fat (*B. Welchii*)	3	*B. mucosus capsulatus*	2
Gram-positive bacilli, slender	3	*Streptococcus hemolyticus*	1
Gram-negative diplococci	2	*Pneumococcus*, Type XXX	1
Gram-negative bacilli, fat (*B. mucosus capsulatus*)	1	*Pneumococcus*, rough, variant	1
Gram-negative bacilli, short and slender (*H. influenzae*)	1		

or bronchioles. With the exception of 1 case, the phagocytic cells were chiefly polymorphonuclear leukocytes. In Case 1, treated with 225 cc. of serum, death occurred on the 13th day of the disease; practically no pneumococci were recovered from the cultures and the majority of the phagocytosed pneumococci were contained in mononuclear leukocytes. In only a relatively few instances were free pneumococci seen in the alveoli in the absence of phagocytosis.

Hydrogen Ion Concentration: The color of the lung suspensions interfered considerably with the determination of the hydrogen ion concentration by the colorimetric method. Furthermore, no attempt was made to protect samples against loss of carbon dioxide. Although the figures listed almost certainly do not represent the actual hydrogen ion concentrations of the different lobes during life, they may, in the absence of data from living cases, serve to furnish an idea as to the relative reactions of the different lobes. In general, the consolidated lobes showed a higher concentration of hydrogen ions than the normal or congested and edematous lobes.

Specific Soluble Substance (SSS): The amounts of SSS in the lungs were large. In general, the greatest amounts were found in the moderately old, consolidated lobes, and the total amounts in the lungs were more frequently large in cases of relatively short duration.

In Case 3, in which death occurred on the 12th day of the disease, the amount of SSS in the consolidated lobes was negligible; in view of the findings in other lungs showing earlier consolidation, it seems probable that it had been present previously in large amounts and had been almost completely removed or destroyed. The same is true for the resolving right lower lobe in Case 9.

Appreciable amounts of SSS were found in the seropurulent pleural fluids in Case 10. The amounts in the blood serum, the urine, the liver and the spleen in Cases 4 and 10 were negligible.

Discussion

Loeschcke,[2] in 1931, published his views on the genesis, course and mechanism of spread of lobar pneumonia, which were based on a systematic gross and microscopic study of about 90 lungs,

fixed *in toto,* from cases of the disease. His division of the disease into five stages, with a description of the transition from one stage to the next, is considered, in this laboratory, to be more acceptable than the early classical descriptions. According to him, there is, first, hyperemia of the alveolar walls, and the alveoli are filled with edema fluid and innumerable numbers of pneumococci. This is followed by the second stage, in which there is migration of the polymorphonuclear leukocytes and, to a less extent, of "alveolar epithelial cells" into the alveoli, with or without extravasation of red blood cells. In the third stage, the alveoli are completely filled with an exudate consisting chiefly of polymorphonuclear leukocytes in a fine meshwork of fibrin threads, few free pneumococci are present, the alveolar walls are very ischemic and, frequently, there is evidence of thrombosis of the small blood vessels. In the fourth stage, the fibrin network shrinks down, the fibrin threads become coarser, the blood vessels in the alveolar walls refill, extravasation of red blood cells into the alveoli usually occurs, the polymorphonuclear leukocytes are markedly decreased in numbers and show extreme degenerative changes, and there is a regeneration of the "alveolar epithelial cells." Finally, in the fifth stage, the polymorphonuclear leukocytes have disappeared and the remaining fibrin is in the form of tiny, compact plugs that contain and are surrounded by "alveolar epithelial cells," which eventually digest the last remains of the pneumonic process.

The findings in the lungs in this series of cases were in accord with Loeschcke's description of the sequence of events and were essentially the same as those described by Robertson and Uhley [3] in human pneumonic lungs, by Robertson, Coggeshall and Terrell [4] in experimental pneumonia in dogs, and by Rhoads and Goodner [5] in dermal pneumococcus infection in rabbits.

Opie [6] studied the antitryptic substance in blood serum which prevents the digestion of fibrin by leukocytes. This antienzyme was found to be active only in an alkaline medium. Kline and Winternitz [7] demonstrated marked impairment in circulation in the consolidated lung, observing that only enough blood is allowed to seep through the vessels to nourish the alveolar walls, with the result that very little serum escapes into the alveoli and the autolysis of the exudate by the leukocytes is unhindered. Lord [8] demonstrated in the lung the presence of a proteolytic

enzyme active in a weakly alkaline medium, and a peptone splitting enzyme active in an acid medium. He expressed the belief that, as the acidity in the consolidated lung increases, the former enzyme becomes inactive and the latter activated. In this series of autopsy cases it was noted that the fibrin plugs in the alveoli adjacent to the septa carrying the bronchi and larger blood vessels were much more dense than those more centrally located. The presence of antiferment in the connective tissue about the septa or the ability of the septal tissues to maintain a slightly alkaline reaction in the contiguous alveoli could, either singly or in combination, explain the relatively slight degree of fibrinolysis noted in the adjacent alveoli.

The outstanding feature of the cultural results was the failure to recover appreciable numbers of pneumococci from the consolidated lobes, regardless of whether the process was early or late. The rapid disappearence of pneumococci from pneumonic processes was long ago noted by Babes [9] and Weichselbaum.[10] Wadsworth [11] reported that he was able to cultivate very few pneumococci from lungs revealing gray hepatization. Similar observations were made by Rosenow,[12] who further showed, by means of lung punctures, that with the onset of resolution in a given area, pneumococci failed to reappear in cases with ultimate recovery, whereas reinvasion in increased numbers occurred in cases with fatal termination. Such was evidently the occurrence in Case 9 in this series, wherein 2 lobes showing very late consolidation contained considerable numbers of viable pneumococci.

The cause of the early loss of viability of the microorganisms is uncertain. Phagocytosis by polymorphonuclear cells apparently is a factor, although destruction of pneumococci or suppression of growth is known to occur *in vivo*, under certain circumstances, in the absence of marked phagocytosis. On the other hand, Robertson and Uhley [3] state that effective destruction of pneumococci does not occur during the polymorphonuclear stage, but rather during the subsequent stage of macrophage proliferation. They succeeded in culturing pneumococci from areas in the former stage of consolidation and noted that the microorganisms, histologically, appeared in good condition, even after phagocytosis by polymorphonuclear leukocytes. From these findings they conclude that phagocytosis

by polymorphonuclear cells does not represent an effective mechanism for disposing of the bacteria, but that widespread destruction occurs during the stage of macrophage proliferation. In this small series of cases marked diminution in the numbers of viable pneumococci occurred during the stage of phagocytosis by polymorphonuclear leukocytes. Although the possibility must be borne in mind that the pneumococci may die more rapidly postmortem in this stage of consolidation than microorganisms in earlier lesions, marked alteration in the cultural picture by postmortem changes seems improbable in those cases examined as early as 3 hours after death; and, since the autopsies performed after longer intervals yielded essentially similar results, it was felt the time factor did not introduce a variable of prime significance.

The relative importance of the polymorphonuclear leukocytes and other factors, specific or non-specific, in causing the early removal of pneumococci from lesions is highly uncertain. There may be considerable variation in the bactericidal power of the polymorphonuclear leukocytes in the lungs in different cases, which is comparable to the individual differences found in the phagocytic activity of cells of the circulating blood of animals by Robertson and Sia,[13] and of man by Sutliff and Rhoades[14] and by Ward.[15] The fact that marked phagocytosis occurred in every case in this series was, by itself, considered noteworthy, for in some instances the amounts of SSS were very large. Rosenow[16] described a soluble substance in pneumococcus autolysates, which inhibited the action of pneumococcic opsonin. Sia,[17] Wadsworth and Sickles,[18] Ward[19] and others have studied the inhibitory effect of SSS upon *in vitro* phagocytosis. Cole[20] established the fact that the soluble substances of pneumonic exudates neutralized the effects of antipneumococcic immune bodies. Virulent pneumococci are not phagocytosed by polymorphonuclear leukocytes except in the presence of immune substances, and it is difficult to understand how such substances remain active in the presence of the very large amounts of supposedly unneutralized SSS. One is led to seek for possible explanations.

Ward[21] showed that, whereas SSS exerted a specific inhibitory effect on phagocytosis, this effect could be neutralized by

amounts of homologous antiserum far less than that required to produce any detectable precipitation and that the addition of an excess of antiserum resulted in the formation of a precipitate, which was ingested by the leukocytes, thus interfering with phagocytosis apparently for mechanical reasons. Furthermore, by *in vitro* experiments with type III pneumococci he showed that homologous convalescent human serum was able to neutralize the inhibitory effect of SSS and sensitize pneumococci in concentrations that produced no precipitation, whereas in similar experiments with antipneumococcic horse serum effective concentrations yielded a precipitate, which was itself antiphagocytic. The findings of Ward suggest that in lobar pneumonia immune substances may be elaborated in sufficient concentration both to overcome the inhibitory action of the SSS and to sensitize the pneumococci although the mechanism of a response that would result in as rapid and as effectual phagocytosis as was observed in this series of cases is difficult to conceive.

Another, and possibly a more rational, explanation is the assumption that some non-specific physicochemical factor in the exudate kills the pneumococci, rendering them more susceptible to phagocytosis. Rich [22] has expressed the belief that such factors are extremely important for the control of infection in many inflammatory foci. The only such factor investigated in this series of cases was the hydrogen ion concentration. As has already been stated, the figures obtained postmortem cannot be assumed to represent the exact pH of the pneumonic exudate *in vivo*. However, since the pH of the consolidated lobes was, in general, conspicuously lower than that of the unconsolidated lobes, it suggests the possibility, advanced by Lord,[23] that a factor in the death of the bacteria may be an increase in the intra-alveolar hydrogen ion concentration, resulting from rapidly dying and autolyzing leukocytes and impaired circulation in the alveolar walls. Lord and Nye [24] have shown that pneumococci are extremely sensitive to slight changes of acidity. The important observation of Rous [25] is noteworthy in this regard. He ascertained that the reaction of granules in living macrophages is pH 3 or less, and called attention to the lethal action that such a high hydrogen ion concentration might be expected

to exert on phagocytosed pneumococci. That changes in the hydrogen ion concentration have an important bearing on the type of cellular reaction and enzymatic activity is emphasized by Rich [22]; thus, indirectly, the viability of the pneumococci may be affected by a lowering of the pH.

With regard to the claim of Robertson and Uhley [3] that the mononuclear phagocyte is actively associated with the removal or killing of viable pneumococci, a study of the cases in this series makes it seem much more likely that the majority of pneumococci are dead and phagocytosed before the mononuclear cells appear in large numbers, that the latter are concerned with the removal of foreign material, chiefly fibrin and dead polymorphonuclear leukocytes, and that the pneumococci seen in mononuclear cells mainly represent those contained in ingested polymorphonuclear cells. Further contributory evidence of the scavenger function of these mononuclear cells is furnished by their marked proliferation in those alveoli, previously noted, that lie adjacent to the septa and that contain dense plugs of fibrin.

Two of the most serious prognostic conditions in lobar pneumonia — positive blood culture and spread of the lesion — appear to be intimately related to lack of uniform consolidation. In the first stage of lobar pneumonia, according to Loeschcke,[2] there is edema and multiplication of pneumococci within the alveoli, to which the body reacts with a rapid mobilization of enormous numbers of polymorphonuclear leukocytes. A uniform, lobar type of consolidation results, and the disease process becomes localized. Isolated foci of alveoli containing pneumococci and edema fluid may persist in areas other than those of consolidation. In these the influx of leukocytes is much more gradual; it is possible that this delay favors the extension of the consolidation and the development of bacteremia. The mechanisms involved in the localization of the pneumonic process are by no means clear. Wadsworth [26] actively immunized rabbits with killed whole pneumococci and found that the introduction of virulent pneumococci into the lungs of animals so immunized did not give rise to an early general infection, but that the microorganisms remained localized in the lungs, where, if the immunization was not of too high a degree, a disease process

was produced that was similar to lobar pneumonia in man. Opie,[27] in a general discussion of inflammation and immunity, stressed the role of hypersensitiveness in bringing antibodies to the site of attack and fixing bacteria at the site of entry so that they cannot enter the blood stream. Lauche [28] has speculatively linked allergy with immunity in the pathogenesis of lobar pneumonia, holding that the character of the disease is the result of hyperergy and an associated partial immunity and that patchy bronchopneumonia develops because of the lack of such sensitivity and in the corresponding absence of as high a degree of immunity. Lauche's hypothesis is not supported by proof. Robertson, Coggeshall and Terrell,[4] however, have shown in experimental pneumonia in dogs that animals with uniform consolidation only rarely develop blood stream invasion and that in animals developing a generalized infection the consolidation is usually patchy. Furthermore, Sutliff and Finland [29] have published statistics indicating a relatively high mortality in cases of bronchopneumonia incited by pneumococci of a given type compared with that in cases of lobar pneumonia of the same type.

The intravenous injection of homologous antipneumococcic serum is quite generally believed to be therapeutically valuable in preventing or controlling the spread of the pneumonic lesion, but its possible action within areas of consolidation has been subject to much controversy. Cole,[30] in an early article on the serum treatment of pneumonia, observed that when treatment was commenced early no extension of involvement of the lung occurred, but that there was no tendency for the already consolidated portions to resolve under the influence of serum therapy. Hunnicutt and Sutliff,[31] on the basis of precipitation tests, concluded that penetration of the consolidated lobe by therapeutic serum occurs, but that there is no evidence to show that the serum reaches the microorganisms in the already formed pneumonic exudate. In studying the circulation in the pneumonic lungs of rabbits, Kline and Winternitz [7] found that trypan blue readily reached the alveoli when instilled into the bronchi but not when injected intravenously; they attributed this failure to the marked impairment of circulation due to the wide distribution of capillary thrombi.

Even were it proved that the serum can penetrate into the

exudate, its effectiveness in the presence of large amounts of SSS would be exceedingly problematical. The establishment of an excess of antibody would seem to be impossible. By determining the amount of antiserum required to precipitate completely the purified polysaccharide in a given dilution of this substance, calculations were made of the amount that would be required to precipitate the SSS from the corresponding concentration in the lung suspensions. On the basis of these figures it was estimated that in Case 10, for example, 60 liters of the highly potent type I antiserum that was used in testing for the SSS would have been necessary to precipitate completely the specific carbohydrate in both lungs, provided, of course, that the SSS in the sample of lung tissue obtained from each lobe represented a fair sample of the total amount in the whole lobe. The possibility must not be overlooked that in cases with ultimate recovery, the concentration of SSS may never reach such a high level. In the light of what is known regarding the inhibitory action of precipitates upon phagocytosis, the establishment of an excess of antibody would appear to be definitely injurious. The mode of action of therapeutic horse serum remains a fertile field for investigation; the fact is clear, nevertheless, that to be effective therapeutic serum must be given early before extensive consolidation has occurred.

It is known that cases of lobar pneumonia may result fatally in spite of the fact that the pneumococcic infection has been largely overcome. This was true in 2 of the 6 cases due to type I pneumococcus, wherein no appreciable numbers of bacteria were cultivated from the lungs. In both Cases 1 and 2 there was no obvious reason for death other than extensive involvement and, in explaining the fatal outcome, the possibility of secondary causes of death resulting from the "toxemia" must be seriously considered, as, for example, profound circulatory disturbances (Atchley,[32] Hitzig *et al.*,[33] and Warfield [34]).

Friedlander bacilli were cultivated from 2 of the 9 cases and were in sufficiently large numbers to be considered as a contributory cause of death. From the experience gained in routine autopsy bacteriology in this laboratory the feeling has arisen that this bacillus and certain other bacteria are usually, if not always, secondary invaders.

Summary

1. Quantitative bacteriological methods have shown that viable pneumococci are extremely scarce in the consolidated lobes of lungs from cases of lobar pneumonia.

2. A quantitative serological method has demonstrated that homologous soluble specific substance is present in such lobes in high concentration and that, as a rule, the moderately old, consolidated lobes contain the largest amounts.

3. Histological examination of sections of the same lobes has shown that the majority of the pneumococci are phagocytosed by polymorphonuclear leukocytes.

4. Certain aspects of the pathology and bacteriology of lobar pneumonia are discussed.

REFERENCES

1. Rudinkoff, I., and Stawsky, Helen. Modified method for staining Gram-positive and Gram-negative organisms in tissue sections. *Arch. Path.*, 1935, **19**, 543–544.

2. Loeschcke, H. Untersuchungen über die kruppöse Pneumonie. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1931, **86**, 201–223.

3. Robertson, O. H., and Uhley, C. G. Changes occurring in macrophage system of lungs in pneumococcus lobar pneumonia. *J. Clin. Investigation*, 1936, **15**, 115–130.

4. Robertson, Oswald H., Coggeshall, Lowell T., and Terrell, Edward E. Experimental pneumococcus lobar pneumonia in the dog. II. Pathology. *J. Clin. Investigation*, 1933, **12**, 433–493.

5. Rhoads, C. P., and Goodner, Kenneth. The pathology of experimental dermal pneumococcus infection in the rabbit. *J. Exper. Med.*, 1931, **54**, 41–50.

6. Opie, Eugene L. Enzymes and anti-enzymes of inflammatory exudates. *J. Exper. Med.*, 1905, **7**, 316–334.

7. Kline, B. S., and Winternitz, M. C. Studies upon experimental pneumonia in rabbits. VIII. Intra vitam staining in experimental pneumonia, and the circulation in the pneumonic lung. *J. Exper. Med.*, 1915, **21**, 311–319.

8. Lord, Frederick T. The relation of proteolytic enzymes in the pneumonic lung to hydrogen ion concentration. An explanation of resolution. *J. Exper. Med.*, 1919, **30**, 379–388.

9. Babes, V. Le rôle du pneumocoque dans l'oedème pulmonaire. *Compt. rend. Soc. de biol.*, 1909, **67**, 725–727.

10. Weichselbaum, A. Ueber die Aetiologie der acuten Lungen- und Rippenzellentzündungen. *Med. Jahrb.*, 1886, N.S. 1, 483–554.

11. Wadsworth, Augustus B. Studies on pneumococcus infection in animals. First paper. *J. Exper. Med.*, 1912, **16**, 54–77.

12. Rosenow, E. C. A bacteriological and cellular study of the lung exudate during life in lobar pneumonia. *J. Infect. Dis.*, 1911, **8**, 500–503.

13. Robertson, Oswald H., and Sia, Richard H. P. Studies in pneumococcus growth inhibition. II. A method for demonstrating the growth-inhibitory and bactericidal action of normal serum-leucocyte mixtures. *J. Exper. Med.*, 1924, **39**, 219–244.

14. Sutliff, W. D., and Rhoades, D. R. The pneumococcidal power of whole blood. I. Description of method. Results in individuals with no history of lobar pneumonia. *J. Clin. Investigation*, 1930, **9**, 43–53.

Sutliff, W. D., and Rhoades, D. R. The pneumococcidal power of whole blood. II. Estimations in lobar pneumonia. *J. Clin. Investigation*, 1930, **9**, 55–68.

15. Ward, Hugh K. Observations on the phagocytosis of the pneumococcus by human whole blood. I. The normal phagocytic titre, and the anti-phagocytic effect of the specific soluble substance. *J. Exper. Med.*, 1930, **51**, 675–684.

16. Rosenow, E. C. Human pneumococcal opsonin and the anti-opsonic substance in virulent pneumococci. *J. Infect. Dis.*, 1907, **4**, 285–296.

17. Sia, Richard H. P. Studies on pneumococcus growth inhibition. VI. The specific effect of pneumococcus soluble substance on the growth of pneumococci in normal serum-leucocyte mixtures. *J. Exper. Med.*, 1926, **43**, 633–645.

18. Wadsworth, Augustus B., and Sickles, G. M. The effect of type-I pneumococcus culture broth on the phagocytic action of type-I pneumococcus serum. *J. Immunol.*, 1927, **14**, 321–328.

19. Ward, Hugh K. Observations on the phagocytosis of the pneumococcus by human whole blood. II. The neutralization of the anti-phagocytic action of the specific soluble substance of antiserum. *J. Exper. Med.*, 1930, **51**, 685–702.

20. Cole, Rufus. The neutralization of antipneumococcus immune bodies by infected exudates and sera. *J. Exper. Med.*, 1917, **26**, 453–475.

21. Ward, Hugh K. An examination of the mechanism of pneumococcus immunity by means of bactericidal measurements. II. The reaction between the anticarbohydrate antibody and type-specific products of the organism. *J. Exper. Med.*, 1932, **55**, 519–530.

22. Rich, Arnold Rice. Inflammation in resistance to infection. *Arch. Path.*, 1936, **22**, 228–254.

23. Lord, Frederick T. Certain aspects of bronchial, pulmonary and pleural disease. *J. A. M. A.*, 1916, **67**, 1981–1984.

Lord, Frederick T. The relation of the pneumococcus to the production of acid in fluid culture mediums and the reaction of the pneumonic lung. *J. A. M. A.*, 1919, **72**, 1364–1365.

24. Lord, Frederick T., and Nye, Robert N. The relation of the pneumococcus to hydrogen ion concentration, acid death-point, and dissolution of the organism. *J. Exper. Med.*, 1919, **30**, 389–399.

25. Rous, Peyton. The relative reaction within living mammalian tissues. II. On the mobilization of acid material within cells, and the reaction influenced by the cell states. *J. Exper. Med.*, 1925, **41**, 399–411.

26. Wadsworth, Augustus. Experimental studies on the etiology of acute pneumonitis. *Am. J. M. Sc.*, 1904, **127**, 851–877.

27. Opie, Eugene L. Inflammation and immunity. *J. Immunol.*, 1929, **17**, 329–342.

28. Lauche, A. Ergebnisse neuerer Untersuchungen über die Pneumokokken und die Pneumokokken-Erkrankungen mit besonderer Berücksichtigung der Pneumonien. *Klin. Wchnschr.*, 1933, **12**, 92–96.

29. Sutliff, W. D., and Finland, Maxwell. The significance of the newly classified types of pneumococci in disease. *J. A. M. A.*, 1933, **101**, 1289–1295.

30. Cole, Rufus. Treatment of pneumonia by means of specific serums. *J. A. M. A.*, 1913, **61**, 663–666.

31. Hunnicutt, T., and Sutliff, W. Penetration of antipneumococci horse serum into the consolidated lung in lobar pneumonia. *Proc. Soc. Exper. Biol. & Med.*, 1932, **29**, 635–640.

32. Atchley, Dana W. Medical shock. *J. A. M. A.*, 1930, **95**, 385–389.

33. Hitzig, W. M., King, F. H., Bullowa, J. G. M., and Fishberg, Arthur M. The circulation in lobar pneumonia with special reference to pulmonary edema. *J. Clin. Investigation*, 1936, **15**, 452.

34. Warfield, Louis M. The treatment of circulatory failure. *J. A. M. A.*, 1936, **106**, 892–895.

THE RÔLE PLAYED BY RHEUMATIC FEVER IN THE IMPLANTATION OF BACTERIAL ENDOCARDITIS *

LOUIS GROSS, M.D., AND B. M. FRIED, M.D.

(From the Laboratories of The Mount Sinai Hospital, New York, N. Y.)

According to Perry,[1] the first recorded clinical and pathological findings in a case of bacterial endocarditis were by Lancisi in 1707. A century later Wells,[2] Corvisart,[3] and Bouillaud [4] clearly described the small excrescences (verrucae) characteristic of rheumatic heart disease. The first attempt at differentiation between these conditions seems to have been made by Ormerod [5] in 1851. In the following year Kirkes [6] described the prolonged form of infectious endocarditis, and in 1885 Osler [7] recorded the important observation that valves which were the seat of "malignant endocarditis" frequently show signs of previous damage. Toward the end of the nineteenth century Kelynack's [8] statistical studies on the frequency with which the valves in bacterial endocarditis show evidence of preexisting lesions marked a considerable advance in this field. In 1903 Glynn [9] reported that 60 per cent of his 65 cases of bacterial endocarditis gave a previous history of rheumatic fever. The succeeding years have been replete with classical contributions to the disease which Libman has termed "subacute bacterial endocarditis" and to the knowledge of which he has made notable contributions.[10-20]

In their excellent monographs on bacterial endocarditis Blumer,[21] Thayer,[22] and Perry [1] emphasized the frequent association and the etiological relation of rheumatic valvular disease to these conditions. In a study of their statistics and of other pertinent reports (Libman,[10] Horder,[23] Billings,[24] Coombs,[25] Clawson,[26] Clawson, Bell and Hartzell,[27] Morrison,[28] Swift,[29] MacCallum,[30] and Davis and Weiss [31]) it becomes clear that previous rheumatic alterations are to be found in at least 50 per cent of hearts that are the seat of bacterial endocarditis. In 1923 Lib-

* Aided by grants from the Lucius N. Littauer and Walter W. Naumburg Funds.
Received for publication May 4, 1937.

man [17] described the simultaneous occurrence of active rheumatic fever and subacute bacterial endocarditis in the same hearts. In 1929 Clawson [32] drew attention to the occurrence of Aschoff bodies in 45 per cent of his 60 cases of subacute bacterial endocarditis and assumed a common etiology for both. In 1933 Saphir and Wile [33] reported 10 cases of subacute bacterial endocarditis in children and adolescents, all of which showed Aschoff bodies in the heart. These authors were of the opinion that these lesions represented two distinct entities. In a recent communication Gross [34] presented evidence that indicates the majority of bicuspid aortic valves seen in adults owe their deformity to a rheumatic process. Since these altered aortic valves are known to be peculiarly disposed to bacterial endocarditis (Paget,[35] Abbott,[36, 37] Osler,[38] Lewis and Grant [39]) the close association of these two conditions was again emphasized. In 1935 VonGlahn and Pappenheimer [40] came to the conclusion that infection of the cardiac valves with non-hemolytic streptococci in rheumatic patients is due to the implantation of the bacteria on unhealed rheumatic vegetations. This view is somewhat similar to that expressed by Königer in 1903.[41] VonGlahn and Pappenheimer's evidence for this conclusion, based on an examination of 26 consecutive cases of subacute bacterial endocarditis, is summarized by these authors as follows:

"1. Vegetations histologically identical with those in rheumatic endocarditis and not containing bacteria are found (*a*) on the same valve as the bacterial vegetations, (*b*) on other valves on which there are no vegetations containing bacteria, and (*c*) on the auricular wall.

2. Aschoff bodies in the myocardium which are taken to indicate active rheumatic disease are found in practically the same proportion of cases of subacute bacterial endocarditis as of uncomplicated rheumatic cardiac disease.

3. Types of bacterial endocarditis other than that due to non-hemolytic streptococci may be engrafted on active rheumatic vegetations. This is a cogent argument against the view that the two types of lesions are a response of different intensity to the same infective agent, unless we dispense with current views as to the histological specificity of the rheumatic reaction."

In a series of publications on the life cycles of various cardiac lesions in the rheumatic heart, Gross *et al.*[42-51] have demonstrated widespread stigmata by which it is possible to recognize an underlying rheumatic process, even though it be extinct. It seemed of interest, therefore, to reinvestigate this question in order to deter-

mine: (*a*) the frequency with which preexisting valvular damage is found in cases of bacterial endocarditis; (*b*) whether or not activity of the rheumatic process is a necessary precursor to the superimposition of bacterial endocarditis; and (*c*) the mechanism or mechanisms that lead to the implantation of a bacterial endocarditis and, in particular, to the rôle rheumatic fever may play in such mechanisms.

Material and Methods

As Libman and Thayer have emphasized, the division of the bacterial endocarditides into the acute and subacute forms based on the duration of the illness is largely arbitrary, inasmuch as on the one hand the clinical course may be prolonged for a number of months in frank cases of bacterial endocarditis which are known generally to run a very acute course (*e.g., Staphylococcus aureus*), and on the other hand death may supervene within a few weeks in such usually protracted conditions as *Streptococcus viridans* endocarditis. In the latter, the shortening of the clinical duration is frequently due to fatal complications. Apart from this, it is often difficult to obtain a precise clinical history of the duration of the clinical phenomena so that one can never be certain that the course alleged to be under 6 weeks may not actually be longer. In spite of these difficulties we have deemed it best to classify our material into the subacute and acute groups for reasons that will become clear.

Subacute bacterial endocarditis, as is well known, is generally associated with organisms of low grade virulence and minimal pyogenic properties, and the duration of the illness is generally longer than 6 weeks. In the 42 unselected cases comprising this first group there was no history of primary bacterial infection elsewhere in the body. Included in this group were 6 cases in which the clinical history was shorter than 6 weeks. In practically all other respects, however, clinically as well as pathologically, these cases could not be distinguished from those in which the duration of the illness was more prolonged. The organisms recovered from the blood stream in these 6 cases were *Str. viridans* in 3, and gonococcus, pneumococcus and anhemolytic streptococcus (gamma) respectively in each of the remaining 3.

In the 28 unselected cases of acute bacterial endocarditis that comprise the second group, the course was very short (average 2 weeks), there were evidences clinically or pathologically of metastatic suppuration, and there existed a definite primary suppurating focus elsewhere in the body. The organisms recovered from the blood stream of these cases were definitely pyogenic, such as *Staph. aureus, Str. haemolyticus,* and so on.

Each group of cases was further subdivided, according to the criteria that are to be described, into "superimposed" cases, *i.e.* those that are implanted on a rheumatic process, and "non-superimposed" cases, *i.e.* those that do not appear to require primary rheumatic damage of the cardiac structures for their implantation.

The clinical histories, bacteriological findings, Wassermann test, electrocardiographic studies and pathological findings in the hearts and kidneys were carefully investigated. The hearts were fixed in 10 per cent formalin-saline * and blocks were cut according to the standardized technique of Gross, Antopol and Sacks.[52] The staining methods were those employed by Gross and Ehrlich.[42]

Macroscopic Features

The macroscopic features of subacute bacterial endocarditis have been clearly described by Harbitz,[53] Litten,[54] Libman,[11, 13] and Orth.[55] The vegetations presented extreme variations in their shape and consistence. In almost all instances they seemed to arise primarily on the closure line of the valves but were attached by a very broad base. In most of the cases the vegetations were fungating and friable. They often spread down the chordae tendineae of the mitral valve, causing rupture of these structures. In some instances the vegetations were flat and consisted of widespread granular irregularities. The color of the vegetations, as described by Libman,[13] may be yellowish, greenish, pinkish or reddish gray, depending somewhat on the organism and the duration of the process.

An important gross feature of the valvular lesion was its bilaterality, *i.e.* the tendency to affect both sides of the leaflet. This is due to the fact that the bacterial lesion frequently spreads around the

* Solution of formaldehyde U.S.P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

tip of the valve from the proximal layer [50] and produces a contiguity infection of the distal layer.

In one heart the center of the anterior mitral leaflet was ballooned out into an aneurysmal sac (Libman [13]) about the dimensions of a small filbert. This sac protruded toward the left auricular cavity. In another heart a similar aneurysmal bulging was present on the right aortic cusp. The aneurysm pointed toward the ventricular cavity and was in part ruptured and eroded.

Five hearts in which the aortic valve was chiefly or exclusively involved showed remarkable burrowing of the lesion into the subjacent myocardium of the interventricular septum or through the septum fibrosum. In 1 of these cases the septum fibrosum showed an aneurysmal bulging toward the right ventricle. In 3 of the cases the burrowing process had ruptured into the pericardial space between the aortic and pulmonic roots, producing a hemorrhagic and fibrinous contiguity pericarditis. In the 5th case * the burrowing process had completely healed and the considerably deepened sinus of Valsalva disclosed a calcified finger-like projection from its base. The projection had apparently eroded the right cusp of the aortic valve. Two cases presented bicuspid aortic valves. The mechanism of their formation and their relation to rheumatic fever and subacute bacterial endocarditis has been reported elsewhere.

Large vegetations on the aortic cusps were almost invariably associated with a characteristic moth-eaten bacterial lesion on the ventricular surface of the anterior mitral leaflet. In most instances this was undoubtedly due to a contiguity infection from the aortic cusps. Such contiguity involvement of the mitral valves was observed in 14 of the 42 cases.

Of the mural endocardial lesions, the auricular bacterial process was the most characteristic and conspicuous. The vegetations presented the same appearance as on the valve. In the healing stages of the auricular bacterial endocarditis the vegetations were converted into small fibrous nodules producing a characteristic shark-skin appearance. Other significant mural lesions consisted

* This case, which will be published *in extenso* by Averbuck and Libman, is one of undoubted subacute bacterial endocarditis which apparently underwent complete healing (with possible bacterial residua deep in the scar tissue). Eight years after the process was considered healed the patient developed a superimposed acute bacterial endocarditis (*Staphylococcus albus*) from which he died.

of: (*a*) generally small vegetations which spread down the chordae tendineae to implant at the apices of the papillary muscles in the left ventricle; (*b*) contiguity spread of the bacterial process below the base of the aortic valve, involving the left side of the interventricular septum for a variable distance (Libman [11]); and (*c*) spread of the lesions up the sinus of Valsalva to involve the aortic root. A characteristic lesion seen in instances of septum fibrosum perforation (not included in this group) is impact implantation of bacterial vegetations on the tricuspid valve or right ventricular wall distal to the septum. The location of such lesions definitely indicates that a fine spray of infected blood from the systolic discharge of the left ventricle initiates the mural vegetative bacterial process. Other mural lesions were not found in the cases comprising this group.

Although the gross appearance of the bacterial lesions in the "superimposed" subgroup was similar to that in the "non-superimposed," the former subgroup could be generally identified by the presence of the macroscopic rheumatic lesions which have been fully described elsewhere. Of considerable aid in determining previous underlying rheumatic damage are the following features: thickening and deformity of the valve at sites not in the immediate vicinity of the bacterial process; thickening and absorption of the chordae tendineae; gross vascularization of the valve (very important); straightening of the free edges of the auriculoventricular valves; aortic commissural fusion; notching of the aortic cusps; very delicate tooth-like prominences of the pulmonic valve free edges; old flat rheumatic left auricular lesions; and pericardial adhesions behind the left auricle. It is of interest to note that 3 of the cases in this group showed advanced button-hole mitral stenosis.

In the non-superimposed group, valve vascularization was never seen grossly; the only gross left auricular endocardial lesions present were those obviously due to the bacterial process, and the portions of the valve leaflets not in the immediate vicinity of the bacterial vegetations were delicate and translucent.

As noted by Harbitz [53] and by Libman,[11] macroscopic indications of healing are not infrequently conspicuous in this condition. This is evidenced by the formation of mounds and ridges of scar tissue around the bacterial process and by the conversion of the

vegetations into irregular, often large, sharply etched, calcific masses not infrequently projecting for a considerable distance beyond the confines of the valve leaflets. The contour of these calcified vegetations was usually distinguishable from secondary calcific changes in rheumatic valves, the latter generally conforming to the topography of the deformed leaflets and rarely projecting an appreciable distance from their surface. They could furthermore be differentiated from the Mönckeberg calcific aortic process [56] in that this latter generally produces more or less smooth nodular excrescences within the cusps and fused commissures, which also remain relatively confined to the valvular tissue.

In several instances fine granular verrucae occupying the characteristic position of the typical rheumatic variety were seen on the closure line chiefly of the mitral and aortic, though sometimes the tricuspid valve. Because of their small size, tawny color, firm consistence, delicacy of contour and distribution on the closure line, one could suspect an associated fresh rheumatic lesion in such cases. This has also been previously observed by Libman.[17] It must be stated, however, that in the absence of these characteristics it does not seem justifiable to make a diagnosis of an active rheumatic process. It will be shown that bacterial vegetations may also exist in the form of small verrucae and furthermore that small non-bacterial vegetations occur in the proximity of bacterial masses even in cases in which there is no evidence of rheumatic infection, past or present.

In 81 per cent of the 42 cases, bacterial vegetations were present on the mitral valve, and in 66 per cent on the aortic valve. In 43 per cent of the cases the mitral and aortic valves were involved together, with or without associated lesions on other valves and left auricular endocardium. The mitral valve, or mitral valve and left auricle, was involved as the only bacterial process in 30 per cent of the cases, and in 25 per cent the aortic valve alone was involved. The tricuspid valve was involved in 6 cases. In 1 case with negative blood cultures and a large interauricular septal defect, the tricuspid valve was the only one affected by the bacterial lesion. In the remaining 5 cases *Str. viridans* was recovered from 3, *Str. haemolyticus* from 1, and the blood cultures were negative in 1. The pulmonic valve (and all the other valves) was in-

volved in 1 case with *Str. viridans* infection. The left auricle was involved in 26 per cent of the cases.

From the entire group of 42 cases, negative blood cultures were obtained in 10, *Str. viridans* in 26, gonococcus in 4, influenza bacillus in 3, and hemolytic streptococcus, anhemolytic streptococcus (gamma) and pneumococcus, each in 1 case. It is of interest to note, in view of the observations by previous workers concerning the predilection for the gonococcus to lodge in the right heart, that this was not observed in our cases. Apart from the fact that the duration of the illness was usually shorter in the cases with gonococcus, pneumococcus and anhemolytic streptococcus (gamma) infection, there were no appreciable differences between these and those due to *Str. viridans*.

Because of the limited number of cases on which these studies have been made, it is hazardous to come to definite conclusions concerning differences in localization of the bacterial endocarditic process as between the superimposed and non-superimposed subgroups. It appears, however, that left auricular involvement and the combination of mitral and aortic lesions was more frequent in the superimposed cases. On the other hand, involvement limited to the aortic valve alone was found more frequently in the non-superimposed subgroup. Apart from this there is a decided similarity between the superimposed and non-superimposed cases as regards the nature, extent and distribution of the bacterial vegetations. It has already been indicated that differentiation between these groups can be made by the associated valvular and other endocardial lesions. In most instances this differentiation can be determined with great accuracy by the microscopic features to be described.

Microscopic Findings

The most conspicuous histological feature of the valvular lesion in subacute bacterial endocarditis, irrespective of whether it was superimposed on a rheumatic process or non-superimposed, consisted of irregular vegetations which were generally of considerable size (Figs. 1, 6, 7, 8). In approximately half of the cases bacteria could be seen within the superficial as well as deep layers of the vegetations. In about one-fifth of the cases the bacteria

appeared to be largely superficially situated, and in the remaining cases they were generally deep. Apart from the bacteria, the vegetations themselves usually appeared to consist largely of platelet material sometimes admixed with fibrin. In about one-third of the cases the platelet material took a peculiar form which somewhat resembled necrotic liver tissue. It is of importance that, in contrast with the acute bacterial vegetations to be described later, polymorphonuclear leukocytes were generally absent from these vegetations and the bacteria often appeared in the form of clumps or packets.

Verrucae which did not disclose bacteria were noted side by side with the bacterial vegetations and occasionally not in their immediate vicinity. In the superimposed cases some of the verrucae appeared to be of rheumatic origin (Fig. 2). However, the fact that somewhat similar verrucae were also observed in the non-superimposed cases in which there was no evidence either in the form of rheumatic stigmata or in the presence of Aschoff bodies that there was present an underlying rheumatic lesion, indicates clearly that one is not justified in accepting such verrucae as evidence of an active rheumatic process. Apparently, then, as a result of the toxic irritative effects of the bacterial infection, non-bacterial verrucae may form on the valves. Their structure may be somewhat similar to rheumatic vegetations.

A destructive process was generally present in the valve. Occasionally this was severe, at other times minimal. Associated with the destructive process there were often present lymphocytes and polymorphonuclear leukocytes, sometimes large mononuclear cells. In approximately 25 per cent of the cases the base of the vegetations showed a reactive process in the form of irregularly distributed large polygonal cells with sharply outlined basophilic protoplasm and fibrocytoid nuclei. At times these cells were multinucleated. It was difficult to determine whether they represent proliferated fibroblasts, macrophages or endothelial cells. We are inclined to agree with Jaffé [57] that the fibroblast is the chief source of these reactive cells. Their number, shape and distribution appeared to occupy an intermediate position between those seen in the rheumatic process and those in the acute endocarditides. In the former, somewhat similar cells are often seen in considerably greater numbers at the base of the verrucae (Fig. 3). On the

other hand, in the 28 cases of acute bacterial endocarditis to be described, these cells were very infrequent and when present showed pyknotic nuclei with rather aborted cytoplasm.

The valvular lesion essentially due to the bacterial process was most clearly seen in the non-superimposed cases. Vascularization was confined to the formation of capillaries. These were often present in the vicinity of a moderately active inflammatory lesion of the valve. In the neighborhood of vegetations, capillaries were sometimes present in extraordinarily large numbers (hypercapillarization) and many were seen to communicate directly with the cardiac chambers. The remainder of the valve leaflet not infrequently showed a scattering of lymphocytes and large mononuclear cells.

In the superimposed cases vascularization with muscular vessels was invariably noted in the mitral valve (Fig. 2) and frequently in the others. Together with the larger vessels, one could generally observe a sheaf of arterioles and capillaries which were obviously a continuation from the ring region. With few exceptions such valves presented fibroelastic reduplication of the proximal valve layers and valve angles, which were often vascularized, formation of collagenous thickening of the valve tip with dipping of the elastic lamellae beneath the scarred mass, reduplication of the endocardial layer of the chordae tendineae with absorption, and widening of the vascularized spongiosa layer. In a few cases evidence of previous rheumatic damage was confined to the presence of a few thick walled vessels within the auricularis layer of the auriculoventricular valves. In such instances, however, there were present elsewhere in the heart other widespread, even though at times inconspicuous, stigmata of an ancient rheumatic infection.

One of the most interesting and significant lesions, which, in its typical form, is apparently confined to subacute bacterial endocarditis, is what we have termed the "spongy" lesion (Figs. 4, 5). This consists of a collection of wide anastomosing cavernous channels presumably originating from dilatation of capillaries in a hypercapillarized area. On serial section these vascular channels were seen at times to communicate directly with the cardiac cavity as well as with other valvular vessels. Although a process somewhat resembling this spongy transformation was noted on rare occasions in very active rheumatic valvular lesions and, as will be

described, in 1 case of acute bacterial endocarditis, the histological appearance of the lesion in those cases differed considerably from the typical large mass of cavernous channels found in subacute bacterial endocarditis. In the rheumatic process, as well as in the case of acute bacterial endocarditis, the spongy lesion was represented by an aborted attempt at such cavernous transformation. Spongy lesions were present in 50 per cent of the cases of subacute bacterial endocarditis. Their site of predilection was in the vicinity of healing vegetations, near chordae tendineae insertions, and in the valve pockets.

When the bacterial process involved the aortic valve to any considerable degree, the inflammatory process was frequently observed spreading through the aortic annulus (Figs. 6, 7), producing at times a contiguity infection of the adjacent pericardium. Because of the considerable necrosis, which occurs in some of the cases, it becomes clear that such a contiguity process may lead to rupture behind or through the sinus of Valsalva with severe infection of the pericardial mantle. This occurred in 3 of the cases and has some clinical significance.

Not infrequently the valve leaflets not directly involved by the bacterial process presented a varying degree of infiltration with mononuclear cells. Although such a mild interstitial valvulitis may be expected to occur in the leaflets of the superimposed cases because of the underlying rheumatic valvulitis, its occurrence in the non-superimposed cases suggests that the infiltration is the result of a toxic irritative process.

When bacterial vegetations did not encroach on the valve rings in the non-superimposed cases, this site was invariably free of newly formed capillaries. In the aortic and mitral rings which normally possess no vasculature (except for occasional isolated capillaries in very few instances), capillarization was not observed in the non-superimposed cases. Similarly, in such instances the ring region was free from inflammatory cells. On the other hand, in the superimposed cases, *i.e.* those in which there was an underlying rheumatic basis, almost all rings disclosed vascularization, capillarization and scattered lymphocytes. Obviously, therefore, this was a valuable index of such previous rheumatic change. However, in the presence of bacterial vegetations in the proximity of the rings, capillarization and sometimes hypercapillarization

was frequent. Together with these, there was also present other cellular manifestations of acute inflammatory involvement.

Subacute bacterial endocarditis *per se* was not infrequently associated with a mild scattering of mononuclear cells in the endocardium of the left auricle. As with the ring lesion, the non-superimposed cases were conspicuous for their absence of capillary penetration and the various endocardial reduplications characteristic of the rheumatic process. Furthermore, in the immediate vicinity of bacterial vegetations there occurred a very florid formation of capillaries which penetrated the endocardium in large numbers to reach the base of the vegetations. This process differs very decidedly from the rheumatic endocardial lesion in which capillary penetration is at a minimum and in which other phenomena such as palisading with basophilic mononuclear cells, and eosinophilic necrosis and swelling of the collagen, are not infrequently present. In superimposed cases with active rheumatic lesions such auricular endocardial inflammatory processes of both types may be seen side by side.

In most instances of subacute bacterial endocarditis the myocardium throughout the heart showed scars in various stages of healing. Apart from this, there was frequently present mild interstitial mononuclear infiltration which was occasionally focal (so-called Bracht-Wachter lesions). Polymorphonuclear leukocytic interstitial myocarditis was rare. Aschoff bodies were found in 9 of the 42 cases (21 per cent). They were all present in the 30 superimposed cases, an incidence of 30 per cent. The significance of this observation will be taken up in the discussion. The blood vessels in the myocardium, particularly the posterior papillary muscle, posterior wall of the left ventricle and interventricular septum, frequently presented fibroelastic alterations. In a few instances thrombosed vessels were noted in the myocardium. In 1 case there was also present a necrotizing arteritis. No bacteria were demonstrable in these lesions.

The horizontal conduction system (bundle of His) presented scattered mononuclear cells and lymphocytes in 3 cases. In 6 additional cases the septum fibrosum showed focal collections of lymphocytes, mononuclear cells and occasionally polymorphonuclear leukocytes with capillarization and the production of muscular walled vessels. In 3 cases the septum fibrosum was acutely

inflamed and necrotic, with abscess formation in 1 instance. It is of interest to note that these lesions of the bundle and septum fibrosum were found only in the superimposed cases.[47]

Similarly, whereas no alterations were encountered in the intervalvular fibrosa or roots of the great vessels in the non-superimposed cases, except in the proximity of a bacterial process, capillarization with scatterings of lymphocytes was observed in the intervalvular fibrosa in approximately one-third of the superimposed cases, and in the great vessel roots in over one-half of the cases. In 1 instance the aortic root showed destruction of the elastica with abscess formation and contiguity spread to the pericardium.

The pericardium showed evidences of irritation in practically every case. The alterations consisted of scattered large mononuclear cells, less frequently lymphocytes and polymorphonuclear leukocytes, thickening of the septa between the fat cells, thickening and hyalinization of the precapillary and arteriolar walls with, occasionally, necrobiotic changes in these, and in a few instances thrombosis. There were absent, however, the characteristic proliferated capillaries, peculiar large bodied mononuclear and binucleated cells and the signet formations seen in Libman-Sacks disease.[58] Moreover, the vascular alterations were not as frequent or as intense as in that condition. There was also absent the tendency for lymphocytes to localize around the lamina propria, and for the development of corkscrew capillaries and larger vessel changes characteristically present in the rheumatic pericardial lesions.[48]

Finally, apart from the lesions described above, 8 of the cases with a duration of 1 year or longer presented certain histological peculiarities. Evidence of healing was very considerable. In several of these cases bacteria were found only with difficulty. They were indistinct in outline, poorly staining and were generally deeply embedded in relative acellular collagenous tissue (Fig. 8). All but 2 of these cases failed to disclose bacteria on culture. Four of these had been diagnosed as in the bacteria-free stage according to Libman's criteria,[11, 13, 14, 16, 17] and 1 was considered healed. In the 2 cases with positive blood cultures the organism recovered was *Str. viridans*. In view of the discussion, which will be taken up subsequently, it is interesting to note that

3 of these 8 cases showed typical Löhlein lesions in the kidneys. One case presented a chronic diffuse nephritis. Six of the 8 cases were superimposed on an old rheumatic valvulitis.

In summary, the 42 cases of subacute bacterial endocarditis could be divided macroscopically, but with greater certainty microscopically, into 30 cases which were superimposed on a rheumatic basis and 12 cases in which there was no underlying rheumatic alteration of the valves. The gross features by which this differentiation into the "superimposed" and "non-superimposed" subgroups can be made have already been described. As regards the distribution of the bacterial vegetations, it need only be noted that left auricular endocardial involvement and the combination of mitral and aortic involvement appeared to be more frequent in the former subgroup, whereas bacterial vegetations confined to the aortic valve were more frequent in the latter. Histologically, the superimposed group could be identified by the presence of the characteristically rheumatic left auricular endocardial and valvular reduplications, valve angle lesions, vascularization and capillarization of the valve rings, leaflets, intervalvular fibrosa, bundle, septum fibrosum and great vessel roots, and by the presence of Aschoff bodies in 30 per cent of the cases. No Aschoff bodies were found in the non-superimposed subgroup. Furthermore, capillarization and inflammatory cell infiltration were absent except in the proximity of a bacterial process. Non-bacterial vegetations were found in both superimposed and non-superimposed cases. Apart from these, the lesions produced by the subacute bacterial endocarditis were as follows: deposition of vegetations which were generally irregular, often large in size, frequently showing blood platelet structure resembling necrobiotic liver tissue and containing, either superficially or deeply, packets of bacteria; reaction at the base of these vegetations in approximately one-fourth of the cases, in the form of peculiar, large, irregularly polygonal mononuclear and sometimes giant cells with basophilic protoplasm; capillarization or hypercapillarization in the proximity of the vegetations; spongy lesions in approximately one-half of the cases; a varying degree of necrosis of the valve leaflet; and the bilaterality of these lesions.

Acute Bacterial Endocarditis

Of the 28 non-selected cases falling in this group, 21 were superimposed on a previous rheumatic process and 7 were non-superimposed. Although this proportion of cases in which there was underlying rheumatic damage is possibly somewhat higher than would be disclosed by more extensive material, it appears that acute bacterial endocarditis does not differ essentially from the subacute form in respect to the important rôle that previous rheumatic damage plays in predisposing the valve to a bacterial process, a point to which little attention has been paid.

So far as the gross appearance of these vegetations is concerned, there is often little to differentiate them from the subacute bacterial endocarditis. On the whole, however, the vegetations tend to be considerably smaller, evidence of healing is generally absent, and destruction of valve tissue may be considerable. The distribution of the bacterial vegetations in these 28 cases was as follows: mitral valve 20 cases; aortic valve 13 cases; combination of mitral and aortic valve 7 cases; tricuspid valve 4 cases; pulmonic valve 1 case; and left auricular endocardium 2 cases. This distribution is strikingly similar to that observed in subacute bacterial endocarditis except for the considerably lower incidence of left auricular involvement. No appreciable differences were observed between the superimposed and non-superimposed subgroups.

The histological differentiation of the acute from the subacute bacterial endocarditis was rendered relatively simple in most instances. Thus, in the acute bacterial cases the vegetations were generally small and flat; the curious liver-like formation was observed in only 2 cases; the vegetations usually consisted of almost solid bacterial masses (Figs. 9, 10), or fibrinous material through which the bacteria were diffusely distributed (not in packets as in subacute bacterial endocarditis), almost invariably reaching the surface of the lesions and often permeated by necrobiotic polymorphonuclear leukocytes; in a number of instances the bacteria could be seen invading the interstices of the collagenous structure of the valve; the underlying valvular tissue showed extreme necrosis and suppuration (Fig. 9), generally far greater than that seen in the subacute group; cellular reaction immediately beneath the bacterial masses was minimal; large mononuclear cells with

somewhat scanter protoplasm than that seen in subacute bacterial endocarditis and with pyknotic nuclei were seen at the base of these bacterial lesions in only 2 instances; capillarization in the proximity of the bacterial vegetations was often minimal and the spongy lesion, in considerably aborted form, was seen in only 1 case. This latter was the most valuable differentiating feature, as was the presence of abscesses in the myocardium.

As in the subacute group, the characteristic rheumatic stigmata readily differentiated the superimposed from the non-superimposed groups (Fig. 10). Thus, the non-superimposed cases of acute bacterial endocarditis were not associated with alterations of the valve ring, intervalvular fibrosa, great vessel roots or left auricular endocardium except in the immediate proximity of a bacterial vegetation. Irritation processes were seen in the pericardium in these as well as in the superimposed cases. Except for a greater tendency to polymorphonuclear leukocytic reaction it was difficult to differentiate the irritative phenomena in the pericardium of this group from the subacute. Aschoff bodies were found in 6 of the 21 superimposed cases (29 per cent).

In summary, acute bacterial endocarditis produces a rapidly destructive valvular lesion, capillarization is at a minimum, vascularization with muscular vessels is absent (except in the superimposed cases), the bacteria are superficially placed on the valvular substance, platelet formation is infrequent, vegetations consist of large solid masses of bacteria and diffuse infiltration with polymorphonuclear leukocytes is not infrequent. The typical spongy lesion was not present in these cases, thus forming a very characteristic differentiating feature. The distribution of bacterial vegetations was somewhat similar to that found in subacute bacterial endocarditis. Ring lesions, as well as other rheumatic stigmas were absent except in the superimposed cases where they could be definitely attributed to the underlying rheumatic process. Myocardial abscesses were frequent in cases of acute, and rare in subacute bacterial endocarditis.

Discussion

It was indicated in the introductory remarks that the duration of the illness and the organism isolated from blood culture are

useful, but by no means certain, methods for differentiating acute from subacute bacterial endocarditis. Certain clinical features, particularly those emphasized by Libman, are of considerable value in attempting to make this distinction. Thus, for example, it has been observed that a bacterial endocarditis in the presence of a primary suppurative process elsewhere in the body generally indicates the acute form of the disease, whereas the absence of such a primary lesion in the presence of hematuria (Harbitz,[53] Libman [11]) is strongly indicative of the subacute form.

A fundamental contribution to the pathological lesions of the kidneys responsible for the hematuria was made by Löhlein [59] in 1910. This observer reported that in chronic "ulcerative endocarditis" there is occasionally found in the kidneys what he considered to be an embolic focal glomerulonephritis. Löhlein's article contains an excellent description and beautiful illustrations of this lesion. These observations were subsequently confirmed by Baehr,[60, 61] and by Gaskell.[62] The former, studying the kidneys from Libman's large collection, noted 1 example of acute diffuse glomerulonephritis, 1 of chronic diffuse glomerulonephritis, and 84 of Löhlein lesions among 91 cases of subacute anhemolytic streptococcus endocarditis with positive blood cultures. Chronic diffuse glomerulonephritis was present in 11 of 34 cases in the bacteria-free stage, and 19 cases showed Löhlein lesions. Baehr shared with Löhlein the belief that the focal glomerular lesions are due to bacterial emboli. In 1932 Bell [63] stated that he found Löhlein lesions in over 50 per cent of his 108 cases of subacute bacterial endocarditis and in 8 out of 125 cases of acute bacterial endocarditis.

In an examination of the kidneys from our cases we have observed the Löhlein lesion in approximately half the cases of subacute bacterial endocarditis. As already mentioned, 1 case with negative blood cultures showed a chronic diffuse glomerulonephritis and 1 case with gonococcus endocarditis presented an acute diffuse glomerulonephritis. Eleven of the cases with negative kidneys were associated with a *Str. viridans* endocarditis. On the other hand, Löhlein lesions were present in 1 case of subacute bacterial endocarditis due to hemolytic streptococcus, in 3 cases due to influenza bacillus, and in 2 cases due to gonococcus. Although most of our cases of acute bacterial endocarditis showed glomerular altera-

tions chiefly in the form of thickening of the capillary loops, in only 2 cases were there found changes which were somewhat similar to the Löhlein lesion. Study of the pathogenesis of these focal glomerular alterations has led us to the conclusion that they represent a focal toxic proliferative and necrotic process, with possibly also thrombus formation. We do not favor the view that the changes are brought about by bacterial embolization. In this respect, therefore, the pathogenesis of this lesion is similar to the so-called Osler node which is considered by Lian, Nicolau and Poincloux,[64] and by Merklen and Wolf [65] to result chiefly from a proliferative endothelial process. The infrequency of Löhlein lesions in acute bacterial endocarditis is possibly due to the short duration of the illness and also to the fact that the bacterial toxins in these acute cases are profoundly necrotizing and lack the ability to stimulate proliferation of the endothelial cells. The milder stimulating properties of the toxic principles from the organisms responsible for subacute bacterial endocarditis (usually *Str. viridans*) lead to a more protracted and less stormy clinical course, to proliferation of subcutaneous capillary endothelium (Osler nodes) and glomerular capillary endothelium (Löhlein lesions) and to the proliferative phenomena in the valves.

Whatever the pathogenesis of the Löhlein lesion may be, its occurrence in acute bacterial endocarditis, and rarely in cases without endocarditis, as well as the finding of diffuse glomerulonephritis in subacute bacterial endocarditis, renders this focal renal lesion a useful but not an absolute means for differentiating these conditions.

In the descriptions given above we have indicated that the histological appearance of the valvular and mural lesions is a valuable aid in distinguishing acute from subacute bacterial endocarditis. We have further shown that it is possible to subdivide the endocarditides into those that are superimposed on a rheumatic process and those that appear to have been deposited on non-rheumatic valves. It is important to bear in mind that in most instances this subdivision can be made with considerable certainty since the superimposed cases present a widespread distribution of unmistakable rheumatic stigmata. It is, of course, conceivable that rheumatic fever may heal with complete restitution to integrity of the tissues without leaving discernible traces.

With our present knowledge, however, we have no means for recognizing such completely effaced lesions, granted this takes place. With this reservation in mind, therefore, we must conclude that in 25 per cent of our cases of both subacute and acute bacterial endocarditis, no underlying rheumatic alteration was present in the valves. In 1 case of subacute bacterial endocarditis there was a co-existing syphilitic lesion of the aortic valve, and in another a calcific aortic sclerosis of the Mönckeberg type. Two cases were associated with bicuspid aortic valves; these were, however, on a rheumatic basis.[34] This still leaves an appreciable proportion of cases without any discernible valvular alterations.

We have already stated that small non-bacterial verrucae are at times found side by side with bacterial vegetations in both superimposed and non-superimposed bacterial endocarditis (Fig. 10). In a few of the superimposed cases these verrucae occupied the typical closure line position and presented the characteristic structure of rheumatic verrucae. In all of these cases the presence of Aschoff bodies in the myocardium confirmed our belief that these verrucae represented a simultaneous active rheumatic infection. In a few cases Aschoff bodies were found in the myocardium but no typical rheumatic verrucae were noted on the valves. In appraising the histological appearance of the verrucae, one is aided by the fact that the typical rheumatic verrucous mass is not of platelet structure; there is a great tendency to its invasion by granulation tissue, chiefly fibroblasts; and the lesions are generally close cropped. In the more active cases, especially in younger persons, the base of the rheumatic verrucae frequently presents large mononuclear cells somewhat similar to those seen in the subacute process except for their considerably greater numbers. Verrucae possessing all these characteristics were found in a minority of the superimposed cases and in none of the non-superimposed cases. In both these groups there were observed small non-bacterial verrucae which, in our opinion, could not be accepted as indicative of an active rheumatic process inasmuch as they lacked many of the characteristics described above, but chiefly because they also occurred in hearts that presented no other stigmata of rheumatic fever. Since the incidence of the more typical rheumatic verrucae was lower than that of Aschoff bodies in the myocardium, and since the Aschoff bodies were present in only 30 per cent of the

superimposed cases, we cannot agree with VonGlahn and Pappenheimer's conclusion that infection of cardiac valves with *Str. viridans* in rheumatic patients is due to the implantation of bacteria on unhealed rheumatic verrucae.

As was previously indicated, Aschoff bodies were found in 45 per cent of Clawson's series of 60 cases, and in 46 per cent of VonGlahn and Pappenheimer's 26 cases. In our own material, Aschoff bodies were present in 9 of the 30 cases of superimposed bacterial endocarditis (30 per cent), in none of the non-superimposed cases, and in 6 of the 21 cases of superimposed acute bacterial endocarditis (29 per cent). Inasmuch as Gross, Antopol and Sacks [52] found Aschoff bodies in only 15 per cent of chronic rheumatic cases, it appears that the higher incidence of these lesions in the bacterial endocarditides is of some significance. For reasons which we have stated above, we cannot agree with VonGlahn and Pappenheimer's view as to the invariable presence of rheumatic activity in these hearts, and therefore believe that if subacute bacterial endocarditis of the non-hemolytic streptococcus type is superimposed on unhealed rheumatic vegetations, this occurs in only a minority of the cases. A further difficulty to the acceptance of their theory is the fact that rheumatic children whose hearts are much more frequently the seat of activity and whose valves also show a much greater frequency of fresh verrucae, are far less apt to develop subacute bacterial endocarditis than are the hearts of adults in whom the chronic rheumatic process is much more frequently found. While it is true that other factors may play a rôle to explain this discrepancy, for example, it may be argued that intercurrent infection may be less frequent in childhood than in the later age periods, there is no definite evidence to support this view. On the contrary, in a study of blood invasion in a variety of conditions, carried out by Lichtman and Gross,[66] it has been shown that streptococcic invasion occurs in approximately 6 per cent of cases. The age of the patient did not appear to play a rôle. Unless some other factor is involved inimical to the formation of bacterial endocarditis in childhood, it would appear that it is the chronic rheumatic valve with its peculiar aptitude to the formation of eosinophilic necrosis of collagen which is more likely to become the seat of a bacterial endocarditis than is the fresh vegetative lesion.

It is well known that a variety of infectious diseases may eventually precipitate an acute attack in an otherwise inactive rheumatic process (Libman [17]). It may well be, therefore, that an implantation of a bacterial endocarditis in such cases may similarly lead to reactivation of a smoldering rheumatic infection. This view, which has been recently suggested by Graybiel and White,[67] appears to be consistent with the facts and affords an explanation for the higher incidence of Aschoff bodies in the superimposed bacterial endocarditides.

There remains to be considered the possible mechanisms that lead to the implantation of a bacterial endocarditis. The great frequency with which underlying rheumatic valvular damage plays a rôle is clear indication of a causal connection between the two, even though, as has been indicated, the presence of fresh verrucae apparently does not adequately account for the susceptibility of the valvular structure. Since the announcement by Koester [68] of the embolic theory of endocarditis 59 years ago, many investigators have assumed that the valvular lesions in bacterial endocarditis were due to the lodgement of bacteria in the vessels of the cardiac valves. The generally rich vascularization of rheumatic valves was considered to afford a plausible explanation for the predisposition of such altered valve leaflets to the implantation (by embolization) of a bacterial endocarditis. Grant, Wood and Jones [69] have stated their reasons for not favoring this embolic theory. We are in full agreement with these authors for the following reasons:

1. In spite of the fact that in rheumatic valvulitis the valve rings are much more frequently vascularized than the valve leaflets, a superimposed bacterial endocarditis occurs frequently on the closure line of the leaflet and rarely at the ring.

2. If bacteria were to lodge in vessels within the valve they would produce an interstitial valvulitis. However, the bacterial endocarditides are not primarily associated with an interstitial valvulitis but appear to arise by implantation on the surface of the leaflets.

3. Furthermore, in true interstitial valvulitis (*e.g.* in rheumatic fever) the lesion is always observed to spread from the ring toward the valve tip, whereas in the bacterial endocarditides the rings are not involved primarily.

4. Twenty-five per cent of our cases were not superimposed on rheumatic valves, and since normal valves do not possess blood vessels,[70] another explanation must be sought to explain this discrepancy.

Furthermore, in considering the remarkable manner in which the incidence of both the acute and subacute bacterial valvular lesions in superimposed as well as non-superimposed cases parallels that of the rheumatic valvular lesions, and also the fact that congenital and acquired cardiac anomalies are well known for their susceptibility to bacterial endocarditis, it seems to us that at least two mechanisms may play a rôle in this connection, *viz.* intrinsic changes in the endocardial tissues (rheumatic deformity, congenital defects) which transform them into a more suitable anchoring ground for transient bacterial invaders; and hydrodynamic factors (tension) which favor implantation of bacteria and aid in the progress of the infection.

As regards the hydrodynamic factors, it has already been shown by Gross and Friedberg [50] that the formation of rheumatic verrucae is considerably influenced by mechanical stress and strain. Thus, it was pointed out that the closure lines of those valve leaflets that are subjected to the greatest pressure changes (left heart) become the seat of verrucous formation. In valve leaflets that have become so stiffened and deformed that they cannot close, a new line of verrucae forms on the site most exposed to eddies and blood currents. Moreover, while the incidence of rheumatic verrucae is usually highest in the left heart, it may become significantly increased in the right heart under conditions that produce increased tension in the lesser circulation. Recent experimental observations by Nedzel [71] have convinced him that "pressor episodes" may induce valvular preparation (adhesiveness) as well as a bacteriemia.

An examination of the data submitted above, concerning the localization of bacterial endocarditis, reveals the following pertinent facts:

1. Bacterial verrucae are apt to localize with preference on the closure line or on the most projecting portions of the valves.

2. In a case of patent foramen ovale with increased tension in the right auricle, the only bacterial valvular lesion found was on the tricuspid leaflet.

3. In cases with perforated septum fibrosum, bacterial vegetations frequently form on the mural endocardium of the right heart opposite the stream of blood ejected from the left heart during systole.

4. The aortic sinuses, which are exposed to considerable tension both during systole and diastole, are apt to show conspicuous progress of the bacterial process, with undermining of the tissues and in some cases rupture into the retroaortic pericardial space.

5. Bacterial endocarditis is apt to occur in the proximity of such congenital cardiac defects as are associated with alterations in the direction or intensity of the blood currents. The endocardium surrounding these defects frequently shows proliferative changes.

These observations clearly indicate that the same hydrodynamic factors which are operative in determining the localization of rheumatic verrucae also serve to anchor transient invading bacteria and to promote the progress of the infection. It is of interest in this connection that these sites of increased tension almost invariably show proliferative changes. It appears possible, therefore, that such proliferative changes either by themselves or because of secondary local intrinsic changes, such as thrombosis (von Jürgensen [72]) or degeneration, afford optimum conditions for bacterial implantation.

Apropos of this latter (intrinsic changes) we are in full agreement with Grant, Wood and Jones [69] that these sites not infrequently present thrombotic lesions during life. Gross and Friedberg [73] have recently shown that the closure line of old rheumatic valves may become the seat of large thrombotic deposits. The pendulous stalks that are occasionally found on such deformed valves seem to indicate their origin from healed thrombotic vegetations. It appears, therefore, that lesions of this type are not necessarily terminal events. Furthermore, the closure line of deformed valves not infrequently presents eosinophilic necrosis of collagen.[50] This, too, may serve as a suitable anchoring ground for bacteria. Apart from rheumatic alterations and non-bacterial thrombotic deposits, proliferative changes in the valvular endothelium also occur in calcific sclerosis of the aortic valve (Mönckeberg type) and in aortic valve incompetency due to syphilis. In

the latter, proliferation of the endocardium is frequently observed on the septal aspect of the left outflow tract immediately below the aortic cusps. We have observed 1 case in which a bacterial endocarditis seemed to be engrafted on such a proliferated area. In addition to these, Friedberg and Gross [58] have shown that in a small per cent of a variety of conditions, including commonly encountered infectious processes, anemias, and so on, small proliferated lesions (eosinophilic multinucleated bodies) may be found on the surface of the valves. They occur more frequently on rheumatic valves than on normal ones. Finally, in a case studied clinically by Libman and reported by Gross,[74] subacute bacterial endocarditis was found to be engrafted on an atypical verrucous endocarditis (Libman-Sacks type). These observations collectively indicate that any process that leads to alterations of the valvular or mural endocardium (thrombotic, proliferative or necrotic) whether these be due to inflammatory changes (rheumatic fever, Libman-Sacks disease) to primarily degenerative processes (Mönckeberg sclerosis), mechanical alterations in the impingement of blood (luetic aortitis, congenital defects), or toxic principles (eosinophilic multinucleated bodies) transforms the local sites into a suitable anchoring ground for bacteria. We do not deny the possibility of implantation on fresh rheumatic verrucae, but believe that this is considerably less important than the factors mentioned above. Nor, indeed, do we believe that the mechanisms discussed are the only ones involved. With our present knowledge, however, it seems justifiable to assume that these play an important rôle even though there may be other contributing factors.

There remains to discuss briefly the possible mechanisms involved in the implantation of bacterial endocarditis in the non-superimposed cases. It has been shown that both non-bacterial thrombotic verrucae [73] and eosinophilic multinucleated bodies [58] also occur on non-rheumatic valves but less frequently than on the rheumatic ones. Furthermore, de Vecchi [75] and others have observed proliferative changes on the valve in a variety of conditions. We have then in this group some of the components in common with those found in rheumatic valves. However, the relative infrequency of these alterations, and the absence of eosinophilic collagen necrosis and other proliferative changes, may explain, at least in part, the lower incidence of bacterial endocarditis

in the non-rheumatic cases. Granted bacterial implantation takes place, the subsequent events will be largely determined by the nature of the organism. If this is of a type that produces a mild stimulating toxin and a prolonged course, proliferative changes will take place in the valve, subcutaneous and renal capillaries, and a smoldering rheumatic infection may be awakened into activity. If the invading organism produces toxins of a drastic necrotizing potency, the clinical course will be short, the local lesions (valves) will be suppurative and necrotic, and proliferation will be minimal. In these cases, however, the factors necessary for the stimulation of the Aschoff body reaction are still operative. It seems to us that a working hypothesis along these lines more adequately explains the similarities as well as differences between acute and subacute bacterial endocarditis.

Summary and Conclusions

There have been described in this report the macroscopic and microscopic findings in the hearts in 42 cases of subacute bacterial endocarditis, and 28 cases of acute bacterial endocarditis. It is shown that while there is no sharp line of distinction between these conditions and a variety of lesions are common to both, certain clinical, bacteriological, anatomical and histological features are of considerable aid in classifying the bacterial endocarditides into these two categories. An important, differentiating histological feature is the spongy lesion that occurs in its typical form, perhaps exclusively, in subacute bacterial endocarditis. Through the presence of certain histological stigmata of rheumatic fever it is possible to recognize those cases of bacterial endocarditis that are superimposed on previous rheumatic damage. In our material 75 per cent of the hearts had been the seat of a previous rheumatic process. In a discussion of the mechanisms involved in the implantation of bacterial endocarditis, reasons are given that indicate activity of a rheumatic infection is not a necessary precursor to the development of bacterial endocarditis. Aschoff bodies were encountered in approximately 30 per cent of the superimposed cases of acute and subacute bacterial endocarditis, and evidence is presented that supports the view that some of these cases were thrown into activity by the superimposed bacterial infection rather than that

the activity of the rheumatic process predisposed to the bacterial endocarditis. Certain mechanisms are suggested as the means by which the endocardial structures are predisposed to a bacterial implantation. These mechanisms include the formation of eosinophilic necrosis of the valve closure line, and thrombotic, proliferative and necrotic changes at these sites. Some of these alterations are brought about by the hemodynamics present in congenital and acquired defects. Others are probably due to inflammatory, toxic or degenerative processes. The endocardial alterations, together with intracardiac tension, seem to predispose the endocardial structures of the heart to bacterial implantation by providing suitable means for anchoring transient bacterial invaders. Some of these mechanisms are present in non-rheumatic valves, but less frequently than in rheumatic valves. It does not appear that the vascularization occurring in rheumatic valves plays an appreciable rôle in the implantation of bacterial endocarditis.

REFERENCES

1. Perry, C. Bruce. Bacterial Endocarditis. John Wright & Sons, Ltd., Bristol, 1936.

2. Wells, William C. On rheumatism of the heart. *Tr. Soc. for the Improvement of Med. and Chir. Knowledge*, 1812, **3**, 373–424.

3. Corvisart, J. N. A Treatise on the Diseases and Organic Lesions of the Heart and Great Vessels. Underwood & Blacks, London, 1813.

4. Bouillaud, J. Traité clinique des maladies du coeur. J.-B. Baillière, Paris, 1835.

5. Ormerod, Edward Latham. The Gulstonian lectures for 1851. *London M. Gaz.*, 1851, **47**, N.S. **12**, 503–512, 529–540, 573–582, 617–621, 661–668, 705–711, 793–800.

6. Kirkes, William Senhouse. On some of the principal effects resulting from the detachment of fibrinous deposits from the interior of the heart, and their mixture with the circulating blood. *Med.-Chir. Tr.*, 1852, **35**, 281–324.

7. Osler, William. The Gulstonian lectures, on malignant endocarditis. *Brit. M. J.*, 1885, **1**, 467–470, 522–526, 577–579.

8. Kelynack, T. N. Notes from the post-mortem room: an analysis of twenty-five cases of malignant endocarditis. *Med. Chron.*, 1896, N.S. **5**, 5–17.

9. Glynn, T. R. The Lumeleian lectures on infective endocarditis mainly in its clinical aspects. *Lancet*, 1903, **1**, 1007–1010, 1073–1077, 1148–1153.

10. Libman, E. On some experiences with blood-cultures in the study of bacterial infections. *Bull. Johns Hopkins Hosp.*, 1906, **17**, 215–228.

11. Libman, E., and Celler, H. L. The etiology of subacute infective endocarditis. *Am. J. M. Sc.*, 1910, **140**, 516–527.

12. Libman, E. Demonstration of the cardiac lesions of subacute bacterial endocarditis. *Proc. N. Y. Path. Soc.*, 1911, **11**, 118–123.

13. Libman, E. A study of the endocardial lesions of subacute bacterial endocarditis, with particular reference to healing or healed lesions; with clinical notes. *Am. J. M. Sc.*, 1912, **144**, 313–327.

14. Libman, E. The clinical features of cases of subacute bacterial endocarditis that have spontaneously become bacteria-free. *Am. J. M. Sc.*, 1913, **146**, 625–645.

15. Libman, E. The clinical features of subacute streptococcus (and influenzal) endocarditis in the bacterial stage. *M. Clin. North America*, 1918, **2**, 117–151.

16. Libman, Emanuel. Observations on subacute streptococcus (and influenzal) endocarditis. *Brit. M. J.*, 1920, **2**, 304–306. (Based on remarks made in the opening discussion of the paper on Subacute Bacterial Endocarditis, presented by Sir Thomas Horder, at the Meeting of the Medical Section of the British Medical Association, Cambridge, July 2, 1920.)

17. Libman, Emanuel. Characterization of various forms of endocarditis. *J. A. M. A.*, 1923, **80**, 813–818.

18. Libman, Emanuel. Subacute Bacterial Endocarditis in the Active and Healing Stages. Practical lectures delivered under the auspices of the Medical Society of the County of Kings, Brooklyn, New York (1923–1924 series), Paul B. Hoeber, Inc., New York, 1925, 246–274.

19. Libman, E. A consideration of the prognosis in subacute bacterial endofever. *Am. Heart J.*, 1925, **1**, 25–40.

20. Rothschild, M. A., Sacks, B., and Libman, E. The disturbances of the cardiac mechanism in subacute bacterial endocarditis and rheumatic fever. *Am. Heart J.*, 1927, **2**, 356–374.

21. Blumer, George. Subacute bacterial endocarditis. *Medicine*, 1923, **2**, 105–170.

22. Thayer, William S. Studies on bacterial (infective) endocarditis. *Johns Hopkins Hosp. Rep.*, 1926, **22**, 1–185.

23. Horder, Thomas J. Infective endocarditis; with an analysis of 150 cases and with special reference to the chronic form of the disease. *Quart. J. Med.*, 1909, **2**, 289–324.

24. Billings, Frank. Chronic infectious endocarditis. *Arch. Int. Med.*, 1909, **4**, 409–431.

25. Coombs, C. F. Rheumatic Heart Disease. William Wood & Co., New York, 1924, 31.

26. Clawson, B. J. An analysis of two hundred and twenty cases of endocarditis; with special reference to the subacute bacterial type. *Arch. Int. Med.*, 1924, **33**, 157–184.

27. Clawson, B. J., Bell, E. T., and Hartzell, T. B. Valvular diseases of the heart with special reference to the pathogenesis of old valvular defects. *Am. J. Path.*, 1926, **2**, 193–234.

28. Morrison, Hyman. Subacute streptococcus endocarditis. *New England J. Med.*, 1932, **207**, 770–775.

29. Swift, Homer F. The heart in infection. *Am. Heart J.*, 1928, **3**, 629–648.

30. MacCallum, W. G. A Text-Book of Pathology. W. B. Saunders Co., Philadelphia, 1932, Ed. 5, 237, 504.

31. Davis, David, and Weiss, Soma. The relation of subacute and acute bacterial endocarditis to rheumatic endocarditis; a study of 66 cases with necropsies. *New England J. Med.*, 1933, **208**, 619–623.

32. Clawson, B. J. The Aschoff nodule. *Arch. Path.*, 1929, **8**, 664–685.

33. Saphir, O., and Wile, S. A. Rheumatic manifestations in subacute bacterial endocarditis in children. *Am. Heart J.*, 1933, **9**, 29–44.

34. Gross, Louis. So-called congenital bicuspid aortic valve. *Arch. Path.*, 1937, **23**, 350–362.

35. Paget, James. On obstructions of the branches of the pulmonary artery. *Tr. Roy. Med.-Chir. Soc.*, 1844, **27**, 162–188.

36. Abbott, Maude E. On the incidence of bacterial inflammatory processes in cardiovascular defects and on malformed semilunar cusps. *Ann. Clin. Med.*, 1925, **4**, 189–218.

37. Abbott, Maude E. On the relative incidence and clinical significance of a congenitally bicuspid aortic valve with five illustrative cases. Emanuel Libman Anniversary Volume, International Press, New York, 1932, **1**, 1–38.

38. Osler, William. The bicuspid condition of the aortic valves. *Tr. A. Am. Physicians*, 1886, **1**, 185–192.

39. Lewis, Thomas, and Grant, Ronald T. Observations relating to subacute infective endocarditis. *Heart*, 1923, **10**, 21–99.

40. VonGlahn, W. C., and Pappenheimer, A. M. Relationship between rheumatic and subacute bacterial endocarditis. *Arch. Int. Med.*, 1935, **55**, 173–185.

41. Köniğer, H. Histologische Untersuchungen über Endokarditis. *Arb. a. d. path. Inst. zu Leipzig*, 1903, **2**, 1–162.

42. Gross, Louis, and Ehrlich, Joseph C. Studies on the myocardial Aschoff body. I. Descriptive classification of lesions. *Am. J. Path.*, 1934, **10**, 467–488.

43. Gross, Louis, and Ehrlich, Joseph C. Studies on the myocardial Aschoff body. II. Life cycle, sites of predilection and relation to clinical course of rheumatic fever. *Am. J. Path.*, 1934, **10**, 489–504.

44. Gross, Louis, Kugel, M. A., and Epstein, E. Z. Lesions of the coronary arteries and their branches in rheumatic fever. *Am. J. Path.*, 1935, **11**, 253–280.

45. Gross, Louis. Lesions in the roots of the pulmonary artery and aorta in rheumatic fever. *Am. J. Path.*, 1935, **11**, 631–646.

46. Gross, Louis. Lesions of the left auricle in rheumatic fever. *Am. J. Path.*, 1935, **11**, 711–736.

47. Gross, Louis, and Fried, B. M. Lesions in the auriculoventricular conduction system occurring in rheumatic fever. *Am. J. Path.*, 1936, **12**, 31–44.

48. Friedberg, Charles K., and Gross, Louis. Pericardial lesions in rheumatic fever. *Am. J. Path.*, 1936, **12**, 183–204.

49. Gross, Louis, and Friedberg, Charles K. Lesions of the cardiac valve rings in rheumatic fever. *Am. J. Path.*, 1936, **12**, 469–494.

50. Gross, Louis, and Friedberg, Charles K. Lesions of the cardiac valves in rheumatic fever. *Am. J. Path.*, 1936, **12**, 855–910.

51. Gross, Louis, and Silverman, Gertrude. The aortic commissural lesion in rheumatic fever. *Am. J. Path.*, 1937, **13**, 389–404.

52. Gross, Louis, Antopol, William, and Sacks, Benjamin. A standardized procedure suggested for microscopic studies on the heart; with observations on rheumatic hearts. *Arch. Path.*, 1930, **10**, 840–852.

53. Harbitz, Francis. Studien über Endocarditis. *Deutsche med. Wchnschr.*, 1899, **25**, 121–124.

54. Litten, M. Ueber die maligne (nicht-septische) Form der Endocarditis rheumatica. *Berl. klin. Wchnschr.*, 1899, **36**, 609–612.

55. Orth. Zwei Fälle chronischer ulceröser Endocarditis. *Berl. klin. Wchnschr.*, 1916, **53**, 1255–1256.

56. Sohval, Arthur R., and Gross, Louis. Calcific sclerosis of the aortic valve (Mönckeberg type). *Arch. Path.*, 1936, **22**, 477–494.

57. Jaffé, R. H. Zur Histologie der Herzklappenveränderungen bei der Endocarditis lenta. *Virchows Arch. f. path. Anat.*, 1932, **287**, 379–392.

58. Friedberg, C. K., and Gross, Louis. The cardiac lesions in Libman-Sacks disease. With a consideration of its relationship to acute diffuse lupus erythematosus. (In press.)

59. Löhlein, M. Ueber hämorrhagische Nierenaffektionen bei chronischer ulceröser Endokarditis (embolische nichteiterige Herdnephritis). *Med. Klin.*, 1910, **6**, 375–379.

60. Baehr, George. Glomerular lesions of subacute bacterial endocarditis. *J. Exper. Med.*, 1912, **15**, 330–347.

61. Baehr, George. Renal complications of endocarditis. *Tr. A. Am. Physicians*, 1931, **46**, 87–95.

62. Gaskell, J. F. The lesions of the kidney in ulcerative endocarditis. *Proc. Roy. Soc. Med.*, 1914, **7**, Pt. 3, 109–118.

63. Bell, E. T. Glomerular lesions associated with endocarditis. *Am. J. Path.*, 1932, **8**, 639–664.

64. Lian, C., Nicolau, S., and Poincloux, P. Histopathologie du nodule d'Osler; étude sur l'endothéliite de l'endocardite maligne a évolution lente. *Presse méd.*, 1929, **37**, 497–499.

65. Merklen, P., and Wolf, M. Participation des endothéliites artério-capillaires au syndrome de l'endocardite maligne lente. *Presse méd.*, 1928, **36**, 97–100.

66. Lichtman, S. S., and Gross, Louis. Streptococci in the blood in rheumatic fever, rheumatoid arthritis and other diseases; based on a study of 5,233 consecutive blood cultures. *Arch. Int. Med.*, 1932, **49**, 1078–1094.

67. Graybiel, Ashton, and White, Paul D. Diseases of the heart; a review of some contributions made during 1935. *Arch. Int. Med.*, 1936, **57**, 791–832.

68. Koester, K. Die embolische Endocarditis. *Virchows Arch. f. path. Anat.*, 1878, **72**, 257–277.

69. Grant, R. T., Wood, J. Edwin, Jr., and Jones, T. Duckett. Heart valve irregularities in relation to subacute bacterial endocarditis. *Heart*, 1928, **14**, 247–261.

70. Gross, Louis. Significance of blood vessels in human heart valves. *Am. Heart J.*, 1937, **13**, 275–296.

71. Nedzel, Alexander. The experimental production of bacterial endocarditis in dogs. *Arch. Path.* (in press).

72. Von Jürgensen, Theodor. Erkrankungen der Kreislaufsorgane. Alfred Hölder, Wien, 1900, 15.

73. Gross, Louis, and Friedberg, Charles K. Nonbacterial thrombotic endocarditis; classification and general description. *Arch. Int. Med.*, 1936, **58**, 620–640.

74. Gross, Louis. The heart in atypical verrucous endocarditis (Libman-Sacks). Emanuel Libman Anniversary Volume, International Press, New York, 1932, **2**, 527–550.

75. De Vecchi, Bindo. The endocarditic process in childhood. *Arch. Path.*, 1931, **12**, 49–69.

DESCRIPTION OF PLATES

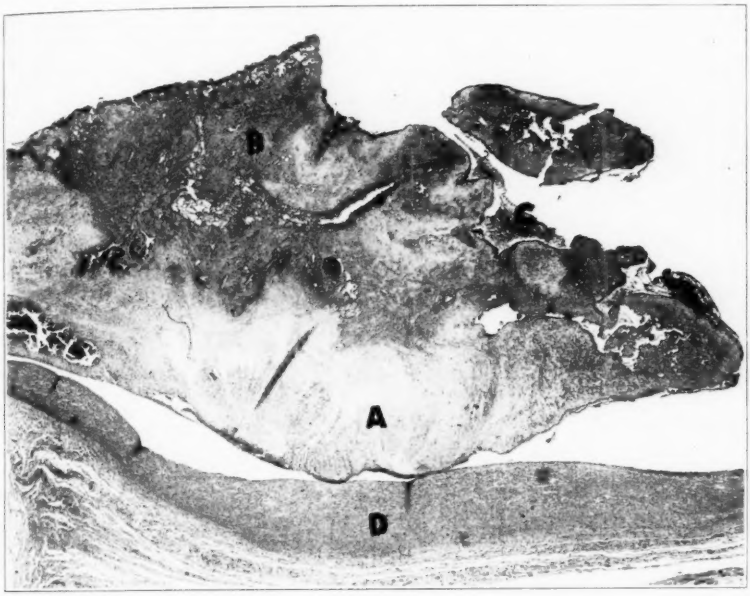
PLATE 104

FIG. 1. Cross section of posterior aortic leaflet from a case of superimposed subacute bacterial endocarditis with negative blood cultures. Low power. Hematoxylin and eosin stain.

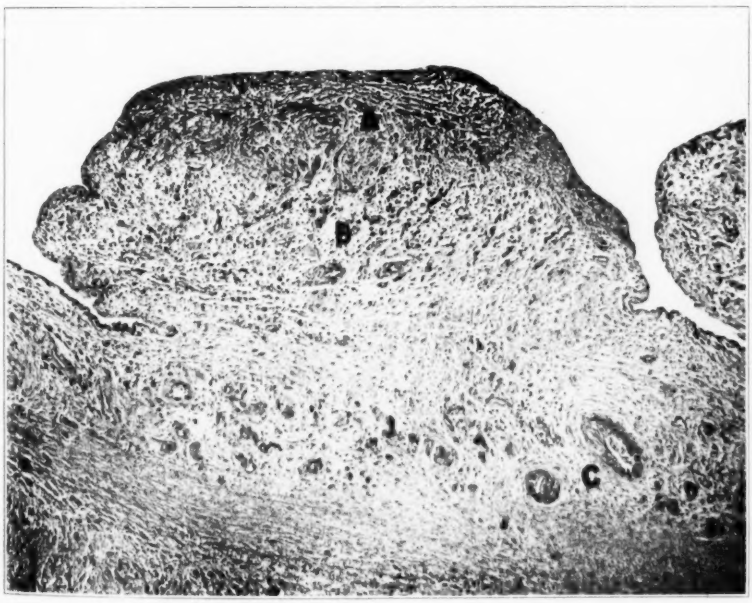
A = fibrosa layer of aortic leaflet; B = huge vegetations consisting largely of platelet structure and containing superficial and deep clumps of bacteria; C = fibrin; and D = aortic root.

FIG. 2. Organizing verrucous lesion on posterior mitral leaflet from a case of superimposed subacute *Str. viridans* endocarditis. Medium power. Hematoxylin and eosin stain.

A = verrucous material; B = extensive organization. (Note numerous capillaries and blood vessels.) C = muscular blood vessels in spongiosa layer of leaflet due to previous rheumatic process.



1



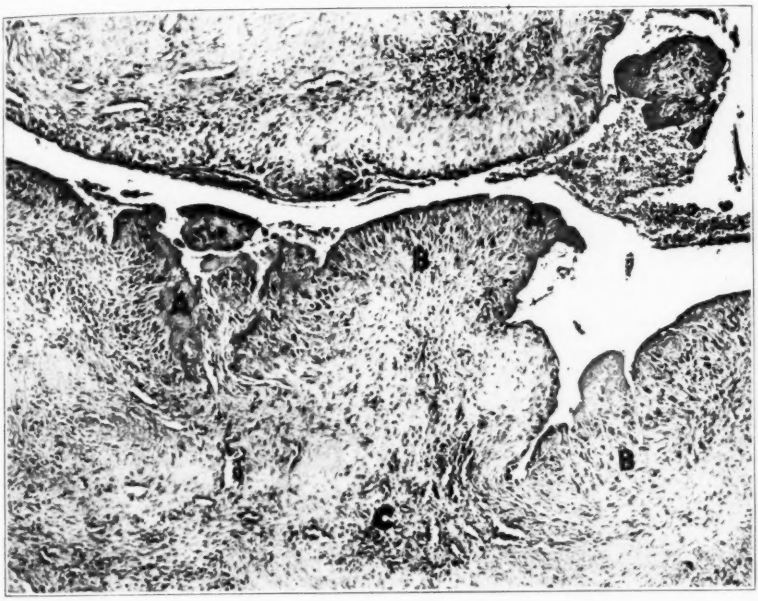
2

UNIV OF MICH.

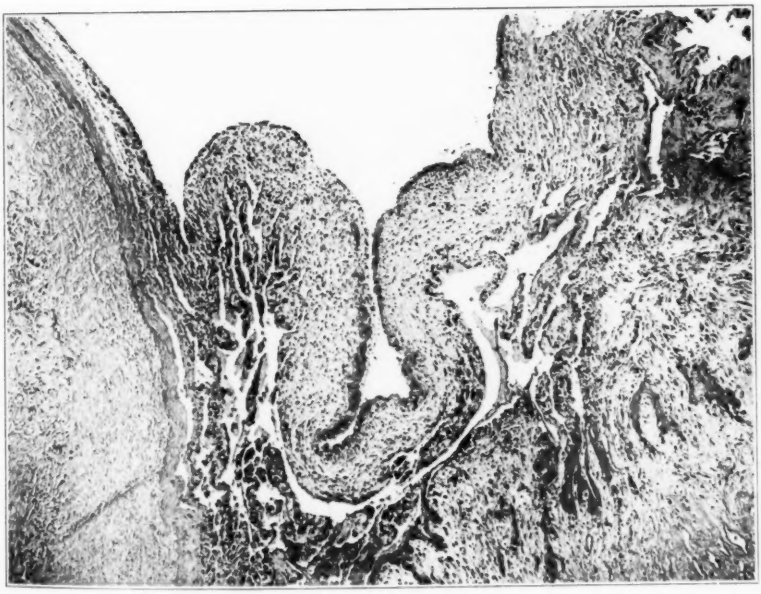
PLATE 105

FIG. 3. Typical rheumatic verrucae on anterior mitral leaflet from an active case of rheumatic fever. Medium power. Hematoxylin and eosin stain. A = close cropped verrucous material; B = active fibroblastic replacement at base. (Note very numerous large polygonal mononuclear cells.) C = intense capillarization of deeper layers of verrucae (part of the rheumatic process).

FIG. 4. Typical spongy lesion situated on the auricularis surface of the anterior mitral valve in a case of non-superimposed subacute bacterial endocarditis with negative blood cultures. Note large intercommunicating cavernous channels and lymphocytic reaction. Medium power. Hematoxylin and eosin stain.



3



4

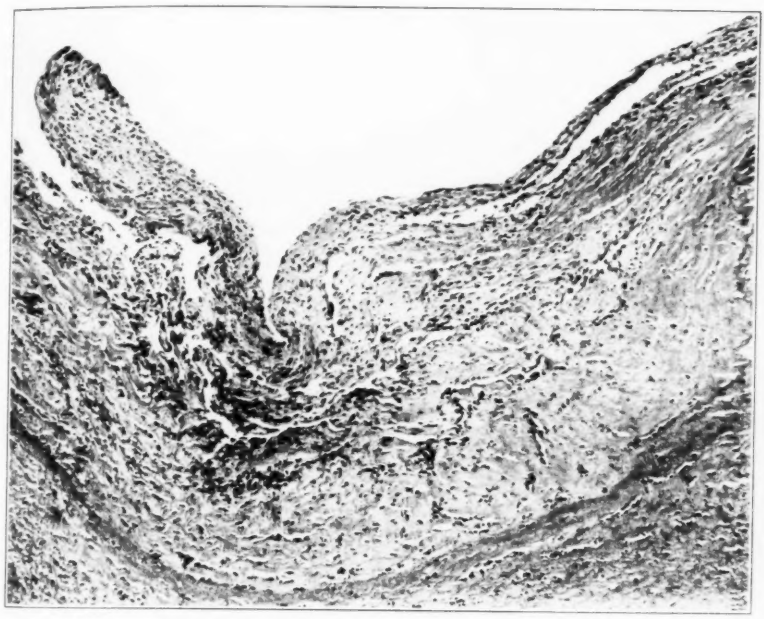
UNIV. OF MICH.

PLATE 106

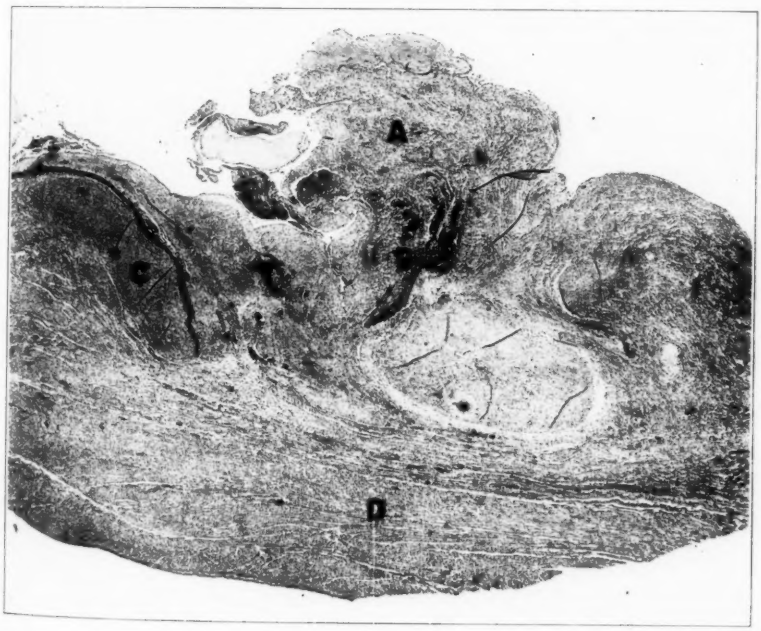
FIG. 5. Typical spongy lesion situated on the auricularis surface of the anterior mitral valve in a case of superimposed subacute bacterial endocarditis (bacteria-free stage). Note large intercommunicating cavernous channels and conspicuous fibrotic transformation. Medium power. Hematoxylin and eosin stain.

FIG. 6. Destructive bacterial process rupturing through base of aortic annulus in a case of superimposed subacute bacterial endocarditis with negative blood cultures. Low power. Weigert's elastic and van Gieson's connective tissue stain.

A = large fungating vegetation; B = ruptured elastic membranes from arterialis surface of annulus; C = lower portion of annulus; and D = myocardium from pulmonary conus.



5



6

Gross and Fried

Implantation of Bacterial Endocarditis

UNIV. OF MICH.

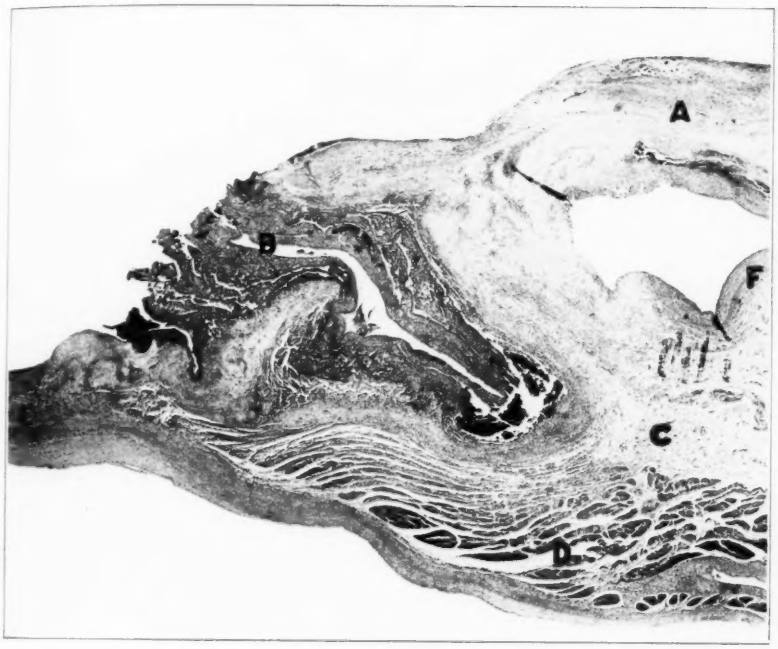
PLATE 107

FIG. 7. Rupture of bacterial process from subaortic angle toward retroaortic pericardial wedge in a case of superimposed subacute bacterial endocarditis with negative blood cultures. Low power. Hematoxylin and eosin stain.

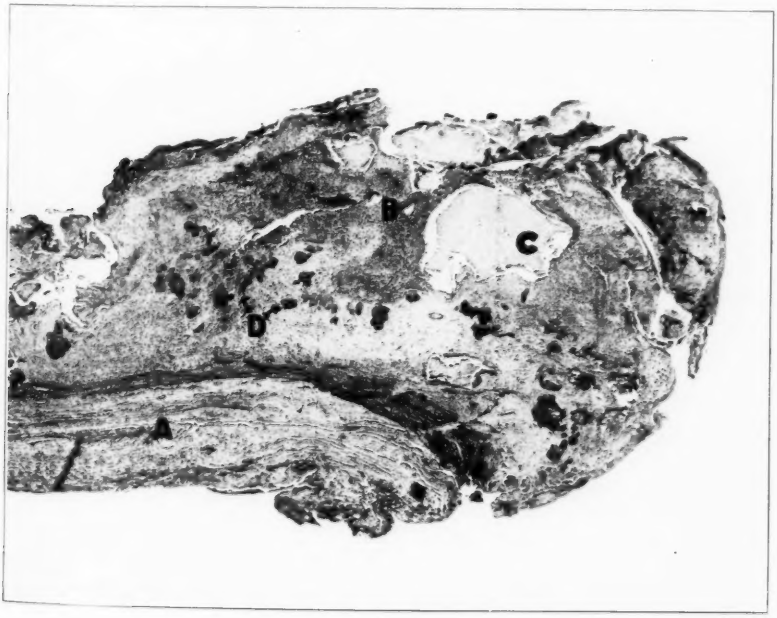
A = right aortic cusp; B = vegetation; C = retroaortic pericardial wedge; D = left auricle; E = mitral valve; and F = aortic root.

FIG. 8. Tip of right aortic cusp from a case of superimposed subacute bacterial endocarditis (bacteria-free stage). Low power. Weigert's elastic and van Gieson's connective tissue stain.

A = fibrosa layer of aortic cusp; B = fine granular material probably representing non-viable bacteria; C = calcific plaque; and D = bacterial clumps embedded in collagenous tissue.



7



8

Gross and Fried

Implantation of Bacterial Endocarditis

UNIV. OF MICH.

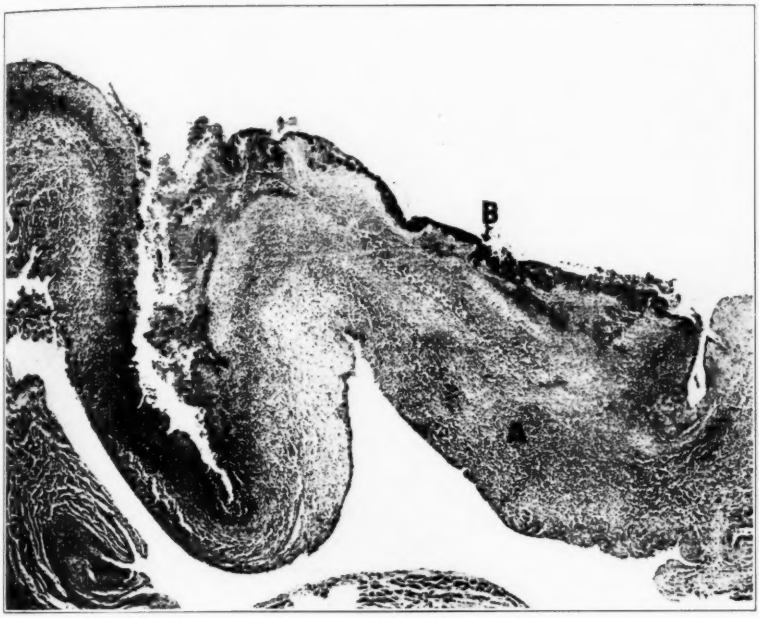
PLATE 108

FIG. 9. Tricuspid leaflet from a case of non-superimposed acute bacterial endocarditis (*Staph. aureus*). Low power. Hematoxylin and eosin stain.

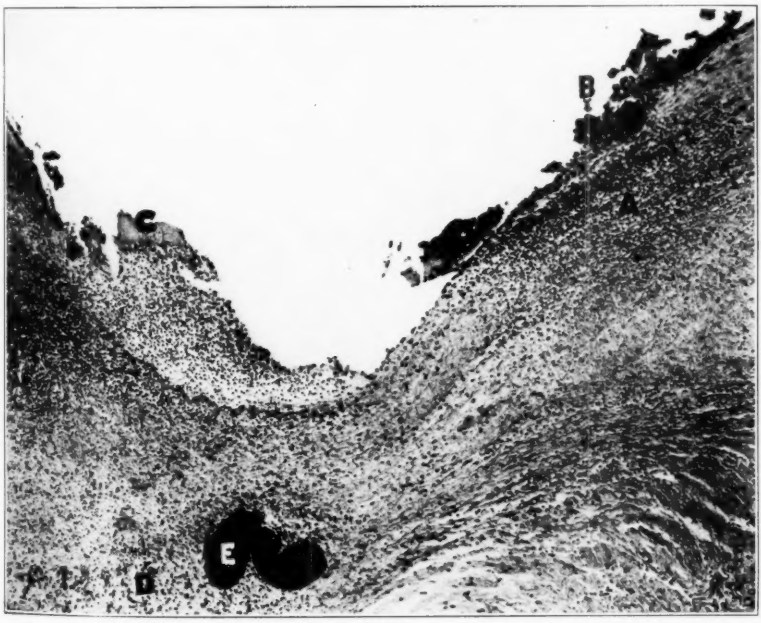
A = intensely necrotic valve leaflet; B = flat superficial bacterial lesion on auricular surface of leaflet.

FIG. 10. Angle of posterior mitral leaflet and left auricle from a case of superimposed acute bacterial endocarditis (*Staph. aureus*). Medium power. Hematoxylin and eosin stain.

A = intensely inflamed and necrobiotic valve leaflet; B = superficial bacterial masses; C = non-bacterial vegetation; D = intensely inflamed ring; and E = large blood vessel (injected) due to previous rheumatic process.



9



10

UNIV. OF MICH.

THE EFFECTS OF COAL SMOKE OF KNOWN COMPOSITION ON THE LUNGS OF ANIMALS *

LUCY SCHNURER, B.S., M.D., AND SAMUEL R. HAYTHORN, M.D.

(From the William H. Singer Memorial Research Laboratory, Allegheny General Hospital, Pittsburgh, Pa.)

The respiratory disease problem, as related to smoke and dust, depends on the degree and kinds of air pollution and varies with the proximity to, and the nature of, the industrial sources in the district. In any large city the problem divides itself into occupational and non-occupational forms of lung damage.

Occupational lung damage depends on exposure to a variety of dusts, organic and inorganic, and varies in degree with the different kinds and concentrations of dust and length of time of exposure. While many dusts may be irritating or produce sensitization, it is now generally conceded that the only inorganic industrial dusts capable of producing disabling fibrotic changes in the lungs are those containing free quartz, mixtures of free quartz with other substances such as coal dust, iron dust, aluminum, and so on, and asbestos. In addition, it is necessary that these dusts be in concentrations greater than 50 million particles per cubic foot containing 5 per cent of free quartz under 10 μ in diameter, or 10 million particles with 35 per cent quartz.[1] Exposures to lower concentrations require longer periods of time than those containing less quartz. If emphasis is placed on the qualifying term "disabling fibrotic lung changes," we concur in this opinion but, based on pathological changes in anthracotic lungs and on experimental results, we take exception to the frequently published statement that carbon in itself is incapable of producing appreciable degrees of fibrosis of the lungs.

In general, non-occupational lung changes are those found in the inhabitants without regard to occupation. They vary with

* Presented at the Thirty-Seventh Annual Meeting of the American Association of Pathologists and Bacteriologists, Chicago, Illinois, March 26, 1937.
Received for publication May 28, 1937.

the concentrations of air contamination of the district and the length of residence.

Coal smoke is the most common type of general air pollution, and its chief particulate constituents are carbon and non-crystalline ash. The silica content of coal smoke, according to our analysis, is far below the levels generally considered injurious, and yet it is a well recognized fact that practically all adult inhabitants of smoky atmospheres show obvious gross and microscopic evidence of particulate deposits, and varying degrees of perilymphatic lung fibrosis.

Reports based on the studies of the relation of lung fibroses to other diseases among industrial groups (see Collis,[2] Sayers,[3] and Lanza [4]), in comparison with the reports made in non-occupational groups and in soft coal miners (see Klotz,[5] Haythorn,[6] Schnurer *et al.*,[7] and Hayhurst [8]), indicate one chief practical difference between the two groups: pulmonary fibroses due to, or associated with, silica dusts in occupational groups predispose to tuberculosis, while fibroses due to pigmentation in non-occupational groups do not. The opposite finding appears to be true with reference to pneumonia. In Pittsburgh, for example, where the inhabitants with non-occupational lung changes far outnumber the industrial employees with more severe forms of lung fibrosis, the pneumonia death rate is usually in the neighborhood of 40 per cent higher than for the Commonwealth of Pennsylvania as a whole. The recent final report for the year of 1935 gave the Allegheny County pneumonia death rate as 121.9 per 100,000 and the State rate as 83.6. This condition has persisted year after year. Although Schnurer has found pneumonia more common in the higher grades of anthracosis than in the less severe, no entirely satisfactory explanation has been found for the locally high occurrence of pneumonia.

It has long been our desire to produce lung changes experimentally which simulate those of the non-occupational types and to study their relation to experimentally produced pneumonias.

Up to the present, the experimental production of the non-occupational types of lung damage, usually called anthracosis but perhaps more correctly termed bituminosis, has generally been unsuccessful save in experiments where free quartz has been

mixed with the carbon. The chief difficulty appears to have been the failure to get sufficient quantities of particulate carbon into the air sacs. Haythorn [6] in 1913 reported the production of fibrous proliferation in the lungs of guinea pigs insufflated with finely powdered, thoroughly dry lampblack. The experiments were carried to the point where several rows of carbon-filled phagocytes had collected in the perivascular lymphatics and had become compressed into spindle shapes by the formation of collagen strands between them. A similar picture was obtained by injecting carbon into the subcutaneous tissues of rabbits' ears. Haynes,[9] cited by Belt,[10] showed by animal experimentation that several dusts usually classed as non-dangerous, including charcoal and colloidal coal, produced proliferative lesions in guinea pigs' lungs indistinguishable from the early lesions of silicosis. Kettle [11] in 1934, in discussing dangerous dusts says, "I have myself seen pleural drift, plaque formation and a moderate degree of fibrosis, that is, the accepted signs of incipient pneumoconiosis with such an inert substance as carbon (India ink)." Opposed to these views are the reports of several French workers. Feil [12] cites Claissi, Josué, Jötten and Arnoldi, all of whom attempted but failed to produce fibrosis in animals exposed to smoke dust. Still others are quoted who claim that lungs blackened by pure coal are never fibrosed. Cummins and Sladden [13] in a comprehensive review of Continental work cite, and are in complete accord with, the results of Stern,[14] whose animal experiments showed that "coal dust alone will cause neither fibrosis nor cavitation."

In America, Willis,[15] reporting on the exposure of guinea pigs to finely sifted coal dust, finds that "fibrosis develops neither within the lungs nor in the tracheo-bronchial lymph nodes after one year of exposure," and concludes that "coal has but slight irritative properties." Lemon and Higgins,[16] speaking of pulmonary fibrosis, briefly dismiss the question with, ". . . and it is universally agreed that carbon dust, unless in large quantities, is of no pathological importance." Cummins and Sladden [13] take the position that anthracosis is a special variety of silicosis, depending on the simultaneous or subsequent accumulation of coal dust in the silica-damaged lung, and Sladden [17] in a more recent paper states that coal dust alone and apart from silica cannot be regarded as a cause of serious fibrosis of the lungs. In Gard-

ner's numerous articles on the effects of the exposure of lungs to various dusts we were unable to find a statement concerning carbon, save that he classified it among the inert dusts. In answer to a direct question he stated that he had obtained slight pneumonitis with an extremely mild proliferation of connective tissue after the inhalation and injection of carbon dust, but he considered this substance inert because the reaction was of such slight degree that it was only visible on microscopic examination. It should be noted here that the articles cited refer to "coal dust" and that our experiments were performed with "coal smoke" and, therefore, have to do only with the particles carried in suspension with the volatile portions of the smoke.

The present report is based on findings that developed in the course of a collaboration of studies made at the Mellon Institute of Industrial Research * on the relative effects of the different kinds of fuels in common use on the lungs. The several kinds of fuel under study were burned in weighed amounts (1000 gm. per hour) continuously in egg stoves for a period of 80 days. Smoke from the flues of each stove was handled separately. One-fourth of the smoke was diverted and mixed with oxygen so that the oxygen content was constantly kept above 19 per cent. The smoke mixtures passed through gas-tight chambers of 60 cubic feet capacity. Four rabbits and 8 rats were placed in each compartment. The controls were placed in a chamber of like construction which was supplied constantly with clean filtered air. For the purposes of this paper, the animals in the bituminous and control chambers are all that need be considered.

The air-gas contents in the chambers were changed completely every 15 minutes. Regular analyses of the gas contents were made. The amounts of sulphur dioxide and carbon dioxide were kept below any percentage reported as toxic, and carbon monoxide

* The experiments were made during the summer and autumn of 1935 as a part of the Anthracite Institute Fellowship at the then uncompleted new Mellon Institute of Industrial Research at Pittsburgh. The engineering features were conducted by members of the research staff of the Institute. The set-up of stoves, the means of adding air and oxygen and of regulating the amounts of flue smoke, the collection of chemical samples and the chemical analyses, were under the direction of H. B. Meller, Head of the Air Hygiene Investigation of the Mellon Institute, and M. A. Yavorsky. A report of the medical phases has been completed by Schnurer and is ready for publication. The animal chambers were borrowed from the United States Bureau of Mines.

did not exceed more than a trace at any time. The animals were kept in the chambers 23 hours out of every 24 (Table I).

TABLE I

Gas-Air Mixtures in Chambers

	O_2	CO	CO_2	H_2S	SO_2
	%		%		%
Maximum	20.8	Trace	10.0	Trace	13.63
Minimum	19.2	"	0.2	"	3.33
Average	20.0	"	0.6	"	7.51

Dust counts were made regularly by Yavorsky, whose results are shown in Table II. Records of the temperature and humidity in each chamber were kept throughout the exposures.

TABLE II

Dust Counts *

Particles per Cubic Centimeter †

	Maximum	Minimum	Average
Control	221	38	125
Bituminous coal	5640	3502	4410

* Owens Jet Counter at 970 X.
† 4410 particles per ccm. = 125 million per cubic foot.

Dr. R. R. Ruppert of the Mellon Institute determined the silica content. The following paragraph is a summary of his report.

"The free silica content of the bituminous coal soot collected in the course of the work was determined, using microincineration to determine the ash in the soot by particle count and the petrographic method of immersion in liquids to find the free silica content of the ash. Using a high magnification, similar to that used in making the dust counts in the course of the tests, the free silica particle content of the soot was found to be 0.8 per cent. That figure multiplied by the dust content of the atmosphere taken in the course of the work gives 0.4 million particles of free silica per cubic foot of air, to which the animals were exposed. That concentration is well below the danger limits, and accord-

ing to any established authority the silica is not present in an amount sufficient to cause fibrosis."

Blood counts were made at weekly intervals. Whenever an animal died an autopsy was performed promptly and tissues preserved for microscopic examination.

Microincineration

Lung sections from animals in each group of experiments were incinerated at 400° C., treated with dilute hydrochloric acid and examined by polarized light under a metallurgical microscope. No highly refractive crystalline particles similar to those found in the incinerated sections of silicotic lungs could be demonstrated. The former positions of the sections were outlined by a fine, gray amorphous ash.

Pathological Effects

In the bituminous chamber 2 rabbits died of bronchopneumonia before the 80 days of exposure were completed. Immediately after the exposure 1 of the 2 remaining rabbits and 6 of the rats were killed and autopsied. The rabbit had a purulent bronchitis and areas of bronchopneumonia. The pleura was diffusely blackened as well as spotted with black pigment and, on section, black blotches appeared on the cut surface throughout the lung tissue. The peribronchial lymph nodes were enlarged and blackened. All of the rats had bronchitis, although no consolidation was found in any of them. The lungs and peribronchial nodes were black or reddish black in color.

Microscopically the findings in the rabbit and rats were similar and separate descriptions would be unnecessary repetition. In all of them the bronchial and bronchiolar goblet cells were swollen and active. A polymorphonuclear and mononuclear cell exudate was often present, and free black pigment and pigment-bearing phagocytes were abundant. The infundibular and alveolar contents varied greatly; some were packed with free pigment and pigmented phagocytes, some contained relatively small numbers of phagocytes, and some were empty. In the areas where pigment was greatest in amount the alveolar walls had collapsed about it and compressed it into masses. The walls of the collapsed air

sacs were congested, thickened, and filled with mononuclear cells and leukocytes. Agglomerations of pigment phagocytes were present in the air spaces and were sometimes fused into multinuclear giant cells. Single pigmented cells were sometimes present in the trabeculae, and collections of them were gathered about the peribronchial, perivascular and subpleural lymphatics. The condition at the end of 80 days was that of a focal interstitial pneumonitis with little fibrous proliferation. In the lymph nodes the pigment was practically all intracellular and the cells were for the most part loosely distributed throughout the lymphoid tissue.

One of the 4 control rabbits died of bronchopneumonia during exposure. The other control animals, killed at the end of the experiment, were either pigment-free or presented a few, widely separated pigment phagocytes in the air sacs.

A rat from each group was killed 103 days after the experiments were discontinued. The control in this instance was essentially normal as far as pigment was concerned. The lungs of the rat from the bituminous chamber were deeply pigmented. Grossly the pleura was studded with black spots having bright, glistening emphysematous centers.

Microscopically these sections resembled those of the animals killed after exposure save that almost no pigment remained free. There were fewer pigment phagocytes in the alveoli and a great many more in the perivascular lymph spaces than in the previous specimens. The blackened patches, seen when the section was held to the light, were made up of groups of alveoli filled with masses of pigmented cells, and the intervening walls showed definite infiltration with phagocytes and clear mononuclear cells (pneumonitis). Fibroblasts in these areas showed a definite increase in number and filamentous strands of collagen, stained blue by Mallory's aniline blue connective tissue stain. In the lymph nodes the pigmented cells were collected in clumps and in fused masses.

The most important results were those in the final rabbit from the bituminous chamber which was killed 429 days after the exposure. Grossly the lungs and pleural surfaces were spotted with discrete black blotches distributed in a pattern similar to that seen in a soft coal miner's lung. The peribronchial lymph nodes were very black and were somewhat enlarged.

Microscopically the findings were milder than those found in

bituminosis and more nearly resembled the condition found in the non-occupational forms of anthracosis. The blackened patches were much like those found in the animals described above except that the foci were more clean-cut, more discrete and more widely separated, while the fibrous proliferation was distinct and unmistakable. The phagocytic collections about the vessels were several rows in thickness and, while they were not compressed into nodules, they were often separated from each other by collagenous strands of varying widths. Local areas of emphysema were often found in the immediately surrounding lung tissue, and there were many single large air sacs, so large that their diameters were equal to the thickness of the whole plaque.

In the lymph nodes the pigment cells had collected in patches and practically no separate unfused phagocytes remained. Under high power examination the patches were found to be unbelievably large fused giant cells, literally packed with black pigment. No collagenous bands similar to those found in the lungs could be demonstrated in or about the pigment masses in the lymph nodes.

Discussion

Rabbits and rats were exposed for 80 days in closed chambers to bituminous coal smoke containing an average of 125 million particles per cubic foot, of which only 0.4 million particles were free silica dioxide. Some of the animals were killed and examined immediately after the exposure, and the rest were returned to the animal house and subsequently examined at various periods for more than a year. The lungs of all of the animals were deeply pigmented, and the amounts and distribution of the black pigment were similar to that of moderate anthracosis. Microscopically the air sacs of the first animals examined were found to be filled with free pigment particles and phagocytes filled with carbon. The phagocytes tended to collect about the bronchioles and in the perivascular and subpleural lymphatics. In addition there were more or less isolated plaques or islands of alveoli completely filled with pigmented cells beneath the pleura and throughout the lung. The animals that were killed later showed practically all of the pigment to be within phagocytes. The collections of pigment in the lymph channels had increased. The plaques of massed carbon cells con-

tained active infiltrative pneumonitis, hyperplastic fibroblasts and a progressively increasing interstitial deposit of collagen. The appearance in the later animals varied from that seen in non-occupational anthracosis to that of the milder forms of bituminosis seen in soft coal miner's lung. Although the collagen was not laid down in whorls, considered by many to be typical of silicosis, the plaques themselves were nodular in structure.

The question then arose as to the nature of the stimulus responsible for the fibrosis. Gaseous elements and amorphous ash could be ruled out on the results of experiments carried out at the same time with coke and anthracite coal smokes in other groups of animals. These fuels produced the same gases and ash contents as soft coal and in amounts that were comparable, yet no pneumonitis or fibrosis was produced. Silica in extremely small amounts and carbon in relatively large quantities remained to be considered. As long as silica was present at all it was not entirely possible to refute the claims of the investigators who maintain the position that where "there is no silica there is no fibrosis." However, the quantity of free quartz was less than one-tenth of that amount stated in the definition of a safe dust. The period of inhalation was only 80 days, and no crystalline particles were found in the sections after incineration. The quantities of carbon were too great to be completely taken care of by phagocytes and, since it is not soluble, it remained in the lungs as a foreign body. That it was not entirely inert is shown by the cellular reaction about it in lungs that were otherwise free from pneumonitis. For these reasons we believe that carbon was responsible for both the pneumonitis and the fibrosis.

Summary

1. An experimental method is described by which finely divided carbon particles in an almost pure state may be successfully introduced into the alveoli of the lungs.

2. Immediately after exposure to the smoke inhalations the lung lesions were those of non-occupational anthracosis.

3. The animals kept under animal house conditions for several months to a year after exposure to smoke developed fibrous reactions about the carbon deposits with the formation of collagen

strands. The resulting lung changes were analogous to those of a milder grade of bituminosis as seen in soft coal miners.

4. The method provides a way of preparing lungs for the experimental study of the actual relation of carbon deposits to the common respiratory infections.

REFERENCES

1. Air Hygiene of America. Engineering Series, 1936, 1, 12.
2. Collis, Edgar L. Milroy lectures (1915). Industrial pneumoconiosis with special reference to dust-phthisis. *Pub. Health, London,* 1915, **28**, 252–259.
3. Sayers, R. R. Silicosis and tuberculosis among miners of the Tri-State District of Oklahoma, Kansas and Missouri. *U. S. Bureau of Mines, Technical Paper* 545.
4. Lanza, A. J. Etiology of silicosis. *J. A. M. A.,* 1933, **101**, 583–584.
5. Klotz, Oskar. Pulmonary anthracosis — a community disease. *Am. J. Pub. Health,* 1914, **4**, 887–916.
6. Haythorn, Samuel R. Some histological evidences of the disease importance of pulmonary anthracosis. *J. M. Research,* 1913, **29**, 259–279.
7. Schnurer, Lucy, Allison, Wesley C., Boucek, Charles M., and Haythorn, Samuel R. Pneumoconiosis in the Pittsburgh district, based on a study of 2,500 postmortem examinations made in Pittsburgh hospitals. *J. Indust. Hyg. & Toxicol.,* 1935, **17**, 294–297.
8. Hayhurst, Emery R. The health hazards and mortality statistics of soft coal mining in Illinois and Ohio. *J. Indust. Hyg. & Toxicol.,* 1919, **1**, 360–367.
9. Haynes, F. Experimental dust inhalation in guinea pigs. *J. Hyg.,* 1931, **31**, 96–123.
10. Belt, T. H. The pathology of pneumoconiosis: A review. *Am. J. M. Sc.,* 1934, **188**, 418–437.
11. Kettle, E. H. The detection of dangerous dusts. *Lancet,* 1934, **1**, 889–890.
12. Feil, A. La conception actuelle de l'anthracoce pulmonaire. *Progrès méd.,* 1930, May 3, 773–781.
13. Cummins, S. Lyle, and Sladden, A. F. Coal-miner's lung. An investigation into the anthracotic lungs of coal-miners in South Wales. *J. Path. & Bact.,* 1930, **33**, 1095–1132.
14. Stern, Gustav. Beitrag zur Frage der Phthisis atra. *Beitr. z. path. Anat. u. z. allg. Pathol.,* 1929, **82**, 178–184.
15. Willis, Henry Stuart. Studies on tuberculous infection. IX. The influence of the inhalation of coal dust on tuberculous infection in guinea pigs. *Am. Rev. Tuberc.,* 1922, **6**, 798–811.
16. Lemon, Willis S., and Higgins, George M. Pulmonary fibrosis: experiments of short duration. *Am. J. M. Sc.,* 1932, **183**, 153–164.
17. Sladden, A. F. The silica content of lungs. *Lancet,* 1933, **2**, 123–125.

DESCRIPTION OF PLATES

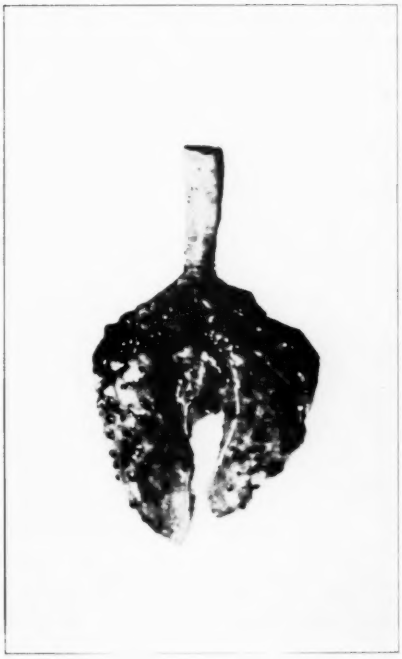
PLATE 109

FIG. 1. Gross appearance of the lungs of a rabbit exposed to bituminous coal smoke for 80 days and kept under animal house conditions for 1 year.

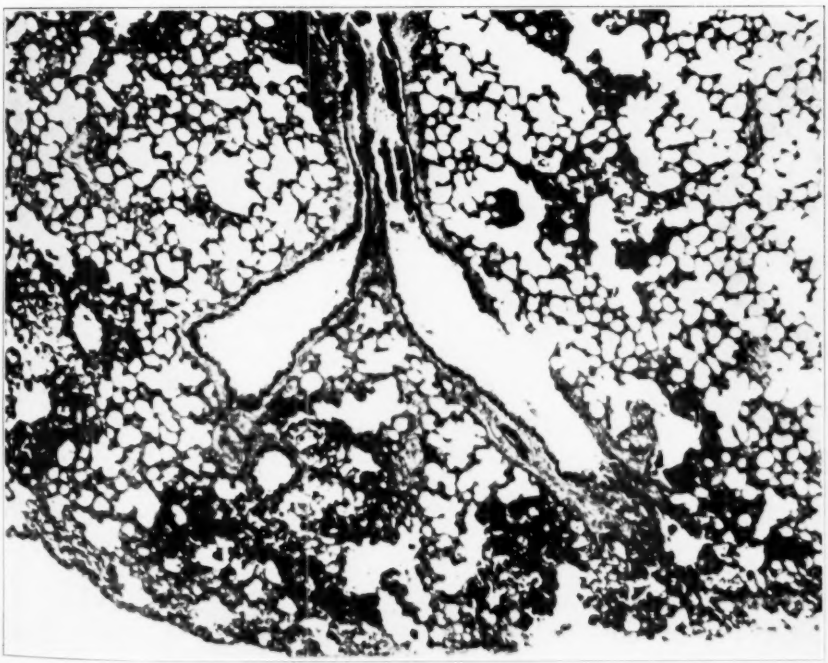
FIG. 2. Gross appearance of the lungs of a rat exposed to bituminous coal smoke for 80 days and autopsied directly after the exposure.

FIG. 3. Low-power section showing the wide distribution of carbon in the lungs of a rat exposed to inhalations of bituminous coal smoke for 80 days and autopsied at the end of the experiment. × 20.

1



2



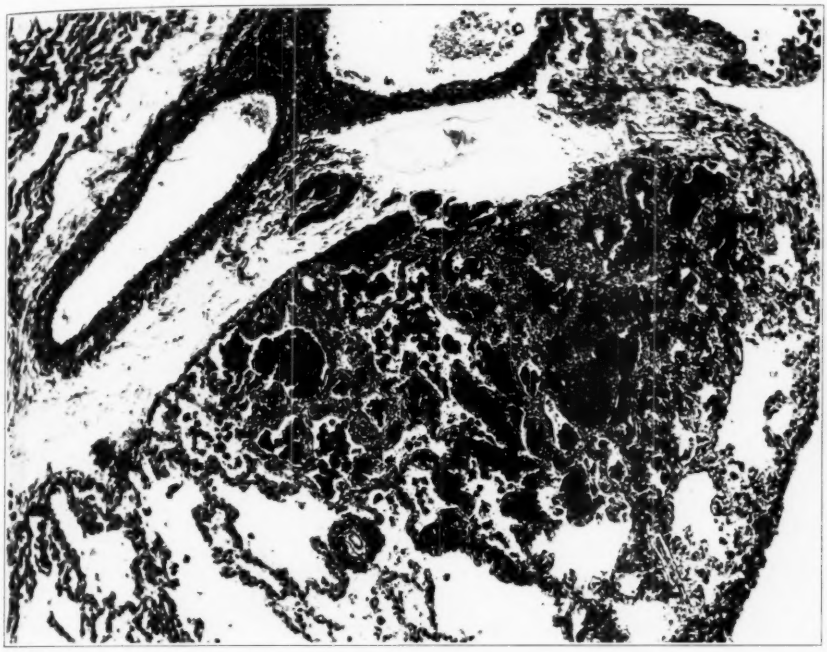
3

UNIV. OF MICH.

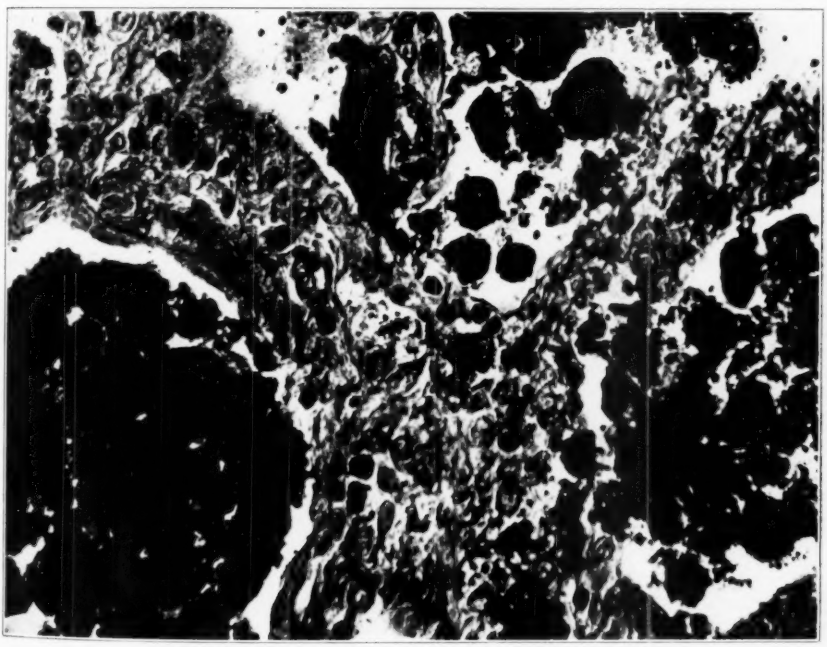
PLATE 110

FIG. 4. Low power section of a rabbit's lung exposed to bituminous coal smoke inhalations for 80 days and kept in the animal house for 1 year. Note the thickened alveolar walls in the pigmented area and the rows of carbon-bearing cells in the perivascular lymph spaces. × 150.

FIG. 5. High power section taken from the nodules shown in Fig. 4 to show interstitial pneumonitis. hyperplasia of the fibroblasts and the deposit of collagen material. × 350.



4



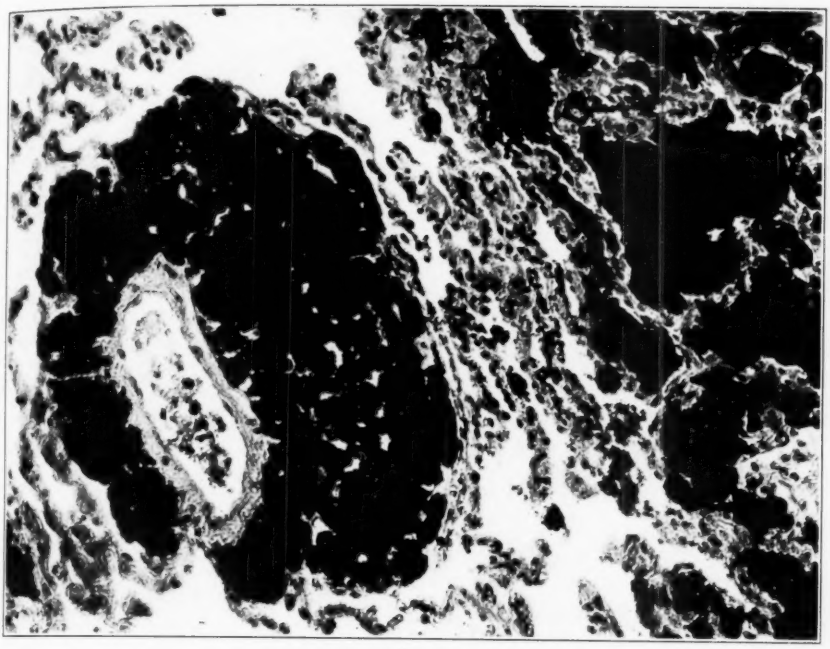
5

UNIV. OF MICH.

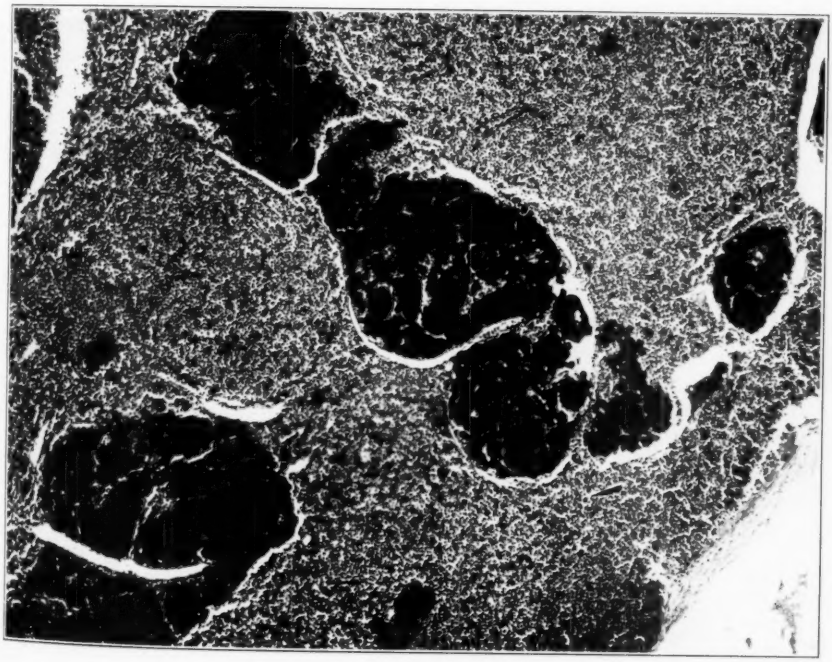
PLATE III

FIG. 6. High power section from the same animal showing the compact pigment deposits in the alveoli and the rows of carbon cells in the perivascular lymphatics. × 200.

FIG. 7. Low power section of peribronchial lymph node showing the accumulation of pigment phagocytes in masses. Many are fused to form large multinucleated giant cells. × 150.



6



7

UNIV. OF MICH.

THE POSTMORTEM ELASTICITY OF THE ADULT HUMAN AORTA. ITS RELATION TO AGE AND TO THE DISTRIBUTION OF INTIMAL ATHEROMAS *

SIGMUND L. WILENS, M.D.

(From the Department of Pathology, New York University College of Medicine, New York, N. Y.)

The association between the elastic properties of arteries and the development of the intimal lesions of arteriosclerosis has never ceased to intrigue interest. The existence of such a relation appears to have been first stressed by Polotebnow [1] in 1868 who observed that a sclerotic femoral artery could be stretched only one-fourth as much as a normal one. The same facts are apparent from the protocols of Wertheim,[2] 1847, and Wundt,[3] 1858, although these authors were more concerned with the physiological nature of arterial elasticity rather than the relation of the latter to disease. That the aorta undergoes a progressive enlargement throughout adult life in both length and circumference is well known. Kaufmann,[4] who made extensive measurements on a large number of aortas, found that the size of the aorta depended chiefly on age and body build. This enlargement with age, it is generally conceded, is associated with a loss of resilience and ability to stretch with traction. Such changes are not necessarily associated with the intimal lesions of true arteriosclerosis but are usually designated as old age phenomena, the result of degeneration of elastin, with alterations in its colloidal properties, and consequently reduced elasticity.

Nevertheless many attempts have been made to demonstrate a relation to the development of intimal atheromas. The various viewpoints are reviewed in surveys by Wells [5] and Aschoff.[6] Perhaps a majority of authors would deny the existence of any other than accidental chronological association. However, the theories of Thoma and his school, that loss of elasticity and weakening of the vessel wall are the primary factors that precipitate all subsequent intimal lesions, have never wanted for ardent supporters

* Received for publication July 2, 1937.

even in recent years. Wells,[5] for example, in a discussion of the chemical changes in arteriosclerosis comments: "It appears that a consideration of the chemical and particularly the physicochemical properties of arteries lends support to the view that arteriosclerosis depends primarily on changes in the elastic tissue that reduce its resilience and lead to arterial dilatation. The subsequent changes seem to be secondary to this yielding of the media." He further quotes Klotz and Beitzke as supporting the views of Thoma in their chief details.

Efforts to measure a possible connection from the physical properties exhibited by arteries have been conducted in two major ways. The degree of elasticity of excised vessels has been measured directly and coefficients of elasticity have been computed from the velocity of pulse wave transmission in the living subject. The early literature on the first of these two methods has been thoroughly and critically reviewed in the extensive monograph of Reuterwall.[7] Only the salient features will be mentioned here.

One group of authors, Moens, Roy, Herringham and Wills, Israel, and Suter (cited by Reuterwall [7]), and more recently Yater and Birkeland,[8] employed narrow strips of aorta of known length and width and measured elongation when these were subjected to variable degrees of traction. Roy [9] graphically recorded elasticity curves and these showed a progressive decline with advancing age and a similarity in form to elasticity curves obtained by stretching the ligamentum nuchae. He also observed a difference in elasticity between longitudinal and transverse strips and noted that the media, when freed of a thickened unyielding intima, often exhibited greater elasticity than when stretched in the intact state. Herringham and Wills [10] found that the greatest loss of elasticity occurred in the fifth decade of life. They stressed the fact that only when intimal lesions were extensive was the reduction of elasticity greater than might be anticipated by age changes alone. Israel [11] found that a variety of disease processes might alter the degree of elasticity exhibited by excised strips of aorta. Suter [12] correlated the loss of elasticity with the enlargement in circumference that occurs with advancing age and also made the interesting observation that the circumference of the aorta when stretched to its maximum was almost constant at all periods of adult life, approximating 11 cm. in the ascending portion.

A variation of this method was to determine the changes in length that occurred when the aorta was freed from its natural attachments (Hiller,[13] Faber,[14] and Scheel and Tendeloo, cited by Reuterwall [7]). The findings of these authors were in general agreement. They found that up to adulthood there was an actual increase in the degree of retraction and thereafter a gradual decline until very late in life when the aorta sometimes was even longer after removal than *in situ*. Both Hiller and Faber thought that intimal plaques exerted little influence on this property of the aorta.

Using other methods some authors (Marey, Roy, Grumach, Zwardemaker, Thoma and his associates, Kaefer, Luck, Poper and Luz, Hurthl, MacWilliam, Strasburger and Pommack, cited by Reuterwall [7]) attempted to simulate conditions as they obtain during life. Unopened segments of arteries, usually medium sized ones, were distended with fluids under increasing pressures and the increase in volume or diameter of the vessel used as an index of elasticity. The results of these experiments, although adding more detail and perhaps more precise measurements, merely served to confirm the major findings of the previous methods. It was from such experiments that Thoma and Kaefer [15] elaborated their theories concerning the pathogenesis of the intimal changes.

The results of this experimentation are open to many serious criticisms if they are used to interpret the functional activities of the arterial system during life. For example, they neglect the role of living irritable cells, particularly smooth muscle, in the reactions of the vessel wall to pressure stimuli. They ignore the influence of the normal attachments and pressure relations of neighboring movable parts on vessel movements. Since the development of reliable methods of estimating the degree of arterial elasticity *in vivo* under natural conditions, notably by Bramwell,[16] and others, the use of excised blood vessels in this connection has been largely discontinued. Nevertheless the results of the *in vivo* experiments have corroborated many of the earlier findings on excised vessels, indicating that elasticity of arteries depends to a large degree on the inert elastic properties of the elastic fibers and that the results of *in vitro* experiments are not necessarily invalid. Moreover Bramwell obtained the same type of

elasticity curves when he applied his technique to excised arteries. The results of both his *in vivo* and *in vitro* experiments were in agreement with many of the early observations of Roy.

Other objections have been raised against the use of excised vessels for the determination of arterial elasticity (Reuterwall [7]). Many difficulties are encountered if absolute measures to conform to the physicist's concept of elasticity are attempted. Arterial elasticity does not seem to obey the same rules that govern the elastic properties of inorganic materials and even of other organic substances. For example, if different methods of measurement are used, quite different quantitative results may be obtained. Moreover, repeated tests on the same segment of artery wall may show quite variable results. The efforts to compare arterial elasticity to that of other substances have not been uniformly successful.

Another criticism is that there is no assurance that the elastic properties may not be so altered by events that transpire before and after death that it becomes impossible to compare the results obtained from different arteries. The actions of toxins, the effects of muscular contraction and postmortem changes have to be taken into account. Nevertheless, there are certain indications that the judicious use of *in vitro* determinations may be of some value. In the first place the results of many different observers have shown striking uniformity, indicating that the postmortem elasticity is no haphazard unpredictable property. Moreover, as already indicated, they agree in many respects with the *in vivo* findings. There is thus some justification for believing that if measurements of elasticity are conducted under identical circumstances, the results should be comparable to each other even if absolute values cannot be obtained and even if the results are not directly applicable to interpretations of the reactions of living vessels.

The application of *in vivo* measurements is limited in that they must be derived from relatively large segments of the vascular tree and cannot be directly associated with development of small intimal lesions. It is generally accepted that the intimal lesions show a definite predisposition to develop earlier and more frequently in certain parts of the adult aorta, namely in the posterior thoracic and lower abdominal areas (Aschoff [17]). Even when the

lesions become more widespread and the earlier pattern is lost, these regions are apt to bear the most advanced ones. If a loss of elasticity commonly precedes the development of these changes, it might be expected that these same areas would show greater losses than for example the more proximal regions of the aorta where atheromas are decidedly less apt to develop.

Moreover, the early work of Roy suggests that at least some of the loss of elasticity that accrues with age is not due to degenerative changes in the elastic fibers of the media but to the restraint exercised by a thickened unyielding intima. Obviously *in vivo* experiments cannot be used to determine the elastic properties of the various layers of the vessel wall, whereas this can readily be accomplished on excised vessels.

With these points in mind, a systematic study of the postmortem elasticity of the aorta was undertaken, first to compare the elasticity of various parts of the same vessel showing different degrees of intimal atheroma, and secondly to determine the role of the intima in producing an apparent loss of elasticity.

Materials and Methods

The aortas of 123 adults, which subsequently showed no microscopic evidence of disease other than arteriosclerosis, were removed in the usual fashion at autopsy. The individuals in this group had died of a wide variety of diseases and no attempt at selection was made other than to ensure a wide age distribution. The vessels were opened by a longitudinal incision along the posterior wall extending between the orifices of each pair of intercostal and lumbar arteries and washed freely in running water to remove blood clot. The adventitia was dissected away as completely as possible with scissors and forceps. The vessels were placed in 0.9 per cent saline and kept in the ice-box until at least 24 hours had elapsed after death in order to allow for a complete loss of smooth muscle irritability. All experiments were conducted within from 1 to 5 days postmortem. Little change in postmortem elasticity occurs during this period even if the aorta is allowed to remain in tap water at room temperature. At the end of such a period the tissue is obviously undergoing decomposition but will exhibit the same elastic properties as when first examined, indi-

cating that this property is not dependent on living cells and that the elastic fibers are quite resistant to postmortem autolysis. Similar observations were made by Roy and subsequently confirmed by many others.

Transverse strips 1 cm. wide were cut from the following areas: 1 from the ascending portion, 2 adjacent ones from the upper descending thoracic portion at the level of the 2nd and 3rd pairs of intercostal artery orifices, 2 adjacent ones from the lower thoracic portion directly proximal to the orifice of the coeliac axis, 1 from the upper abdominal region immediately distal to the orifices of the renal arteries and 1 from the lower abdominal region just above the bifurcation. All the strips represented the complete circumference except the ones taken from the ascending portion, which were sometimes incomplete. Four longitudinally directed strips, 1 cm. wide and about equal in length to the transverse thoracic ones, were cut from the midthoracic region of the descending aorta, 2 from the posterior half and 2 from the anterior half. In addition a single transverse strip 1 cm. wide was cut from the pulmonary artery just proximal to the obliterated ductus arteriosus.

The elastic property of each strip was measured in the following manner. One end of the strip was fastened by a small clamp with serrated jaws supported on a ring stand. A total of 300 gm. of weight was attached and suspended by means of a second clamp from the lower end of the strip for 1 minute. The stretched length was then measured to the nearest 0.05 cm.: 295 gm. were removed and the strip allowed to shorten for another minute and the length again measured. The degree of shortening that occurred when a strip stretched with a load of 300 gm. for 1 minute was subsequently allowed to retract against a load of 5 gm. for 1 minute was used as the measure of elasticity. This was expressed by dividing the actual shortening by the maximum stretched length. This figure multiplied by 100 gives the percentage of shortening.

It must be admitted that this is a highly arbitrary method of estimating elasticity and one that makes no attempt to imitate the natural conditions under which arteries are distended during life. For the purposes of this study a simple test which could be done rapidly under constant conditions on a large number of

segments was required. A 300 gm. weight was found to be great enough to overcome muscular rigor or contraction and to produce almost maximum stretching without tearing or traumatizing many of even the more delicate strips. Measurements on damaged strips were discarded. The final 5 gm. weight was used to keep the strip taut and prevent errors in measurement due to curling. The minute intervals were long enough to allow the tissue to come to equilibrium and short enough to prevent excessive drying. The tissue was allowed to reach room temperature before it was stretched. The degree of retraction rather than extension was used in order to eliminate unrecoverable yielding of the tissue not related to its elasticity. A stretched strip of aorta never quite returns to its original length. Only one test was performed on each strip. Where 2 adjacent strips were cut, 1 was tested in its intact state and the other after the intima had first been detached.

The method used for expressing elasticity requires some justification. Triepel,[18] studying the elasticity of a variety of animal tissues, elaborated a formula for the coefficient of elasticity in which this value varies inversely with the area of the cross section of the tissue tested. With lesser degrees of traction this quite likely holds true for aortic tissue as well, *i.e.* the resistance offered by aortic tissue to slight degrees of traction may increase with the thickness and width of the tissue. When, however, the tissue has been stretched almost to its maximum, as with a 300 gm. weight, subsequent retraction is simply due to the ability of straightened elastic fibers to recoil when released and is independent of the total number or amount of elastic fibers. This can be readily verified by subjecting 2 adjacent strips of aorta of equal length and thickness, but 1 strip 0.5 cm. wide and the other 2 cm. wide, to a 300 gm. traction. Both strips will stretch to an equal length and when the load is reduced to 5 gm. the degree of retraction is the same in both the wide and the narrow strips. In other words, the degree of retraction as measured under the conditions described is *not influenced* by the area of the cross section of the tissue. One cm. wide strips were used in order to limit rather narrowly each area to be tested and to facilitate the computation of intimal weight as described below. In further support of this contention are the observations of Yater and Birkeland [8] who employed strips of constant width and found that the elasticity was entirely inde-

pendent of the thickness. The use of Young's modulus * to express elasticity is also not indicated for the same reason since the area of cross section is again a factor in this formula. Krafka [19] has calculated Young's modulus from the data of a number of other investigators and found that it showed little constancy and did not vary regularly with age.

The degree of intimal sclerosis was estimated by stripping the intima from the underlying media, obtaining the moist weight of the detached membrane to the nearest milligram. This weight divided by the length of the strip yielded the average weight of the intima per sq. cm. of surface for that particular portion. That the intima of the aorta is quite readily detachable from the underlying media was first noted by Roy. In most instances the separation is readily accomplished by peeling away the intima as an intact membrane which includes the entire bulk of any intimal plaques. The underlying media presents a smooth pale yellow surface. With care the separation of small strips can be accomplished with reasonable accuracy, experience showing when the underlying media is being torn since this tissue separates as thin elastic shreds and leaves a rough surface. Microscopic sections through such denuded vessel walls show a reasonably intact inner medial surface and sections of the detached intima regularly fail to reveal the presence of attached smooth muscle cells or elastic fibers. The only difficulties were encountered in cases of young individuals with delicate smooth intimas which were best separated by scraping with a knife blade, and in advanced intimal lesions where the plaques had encroached upon and become more firmly adherent to the media. But even in these instances it was felt that the weight of the intima could be obtained without undue error.

This gravimetric method of estimating the degree of intimal involvement over a small area offers obvious advantages to the usual subjective method of grading the lesions by mere inspection. The drawbacks to the method are that it measures diffuse intimal thickening not necessarily related to the atheromas and gives no exact idea of the type and nature of the lesions. As expected, the

* Krafka uses the formula $\frac{FxL}{axdl}$ to express Young's modulus where F is the weight applied as traction, L the length of the test strip, a, the area of the cross section, and dl, the increase in length.

weight of the intima per sq. cm. showed a rough correlation to the macroscopic severity of intimal change. The delicate intima of young persons showing no plaque formation was about 20 mg. per sq. cm. The vast majority of normal intimas of all persons was in the range of 20 to 40 mg. per sq. cm., although occasionally, it was as high as 60 mg. per sq. cm. The presence of small lipoid streaks altered the weight only slightly. The presence of hyaline plaques produced a significant rise from 60 to 100 mg. per sq. cm., whereas the presence of grossly appreciable calcium deposits caused a sharp rise usually well above 100 mg. per sq. cm. It is thus seen that the more advanced lesions, namely, those showing hyalinization and calcification, were the ones that showed greatly augmented intimal weights.

Results

Relation of Age to Postmortem Elasticity of the Aorta

In Table I the aortas are grouped by age in decades and the postmortem elasticity of the intact aorta is shown at the various levels tested. In the third decade the elasticity is fairly uniform at all these points and in both transverse and longitudinal planes. The posterior thoracic portion longitudinally and the lower abdominal segments have slightly lower values. This is a point of interest when it is recalled that these are precisely the areas in which the earliest atheromatous deposits are usually observed. With successive age groups there is a constant, fairly uniform decline in elasticity throughout life. The loss in longitudinal elasticity, particularly that of the posterior thoracic portion and of the abdominal portions transversely, proceeds more rapidly in the earlier decades of life and is always more severe than elsewhere. Later in life the other areas show greater reductions so that in the oldest age group there is less difference between the various levels tested.

The pulmonary artery in young persons exhibits less inert elasticity than the aorta. It too shows a progressive decline throughout life but one of much lesser degree so that in the oldest age group it has retained considerably more elasticity than any part of the aorta.

TABLE I

Relation of Mean Postmortem Elasticity of Intact Aorta to Age

Area	Direction	20–29 yrs.		30–39 yrs.		40–49 yrs.		50–59 yrs.		60–69 yrs.		70–85 yrs.	
		No.	Mean % **	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
Ascending *** thoracic	Transverse	8*	37.4 ±0.66	9	34.0 ±0.64	10	31.7 ±0.68	12	25.8 ±0.97	10	23.0 ±0.94	11	15.8 ±0.93
Upper descending thoracic	Transverse	15	37.0 ±0.46	13	33.7 ±0.52	23	29.7 ±0.42	21	27.0 ±0.58	21	21.5 ±0.48	18	16.2 ±0.63
Lower descending thoracic	Transverse	14	38.2 ±0.46	12	34.3 ±0.63	22	29.8 ±0.38	21	26.6 ±0.71	20	21.1 ±0.51	16	16.1 ±0.54
Midthoracic descending anterior	Longitudinal	12	33.5 ±0.51	12	28.5 ±0.51	22	22.9 ±0.46	22	21.4 ±0.54	20	17.2 ±0.53	17	14.2 ±0.43
Midthoracic descending posterior	Longitudinal	11	30.3 ±0.75	12	26.8 ±0.52	21	19.6 ±0.41	22	17.6 ±0.53	20	12.7 ±0.53	17	11.2 ±0.49
Upper abdominal	Transverse	13	36.4 ±0.56	11	31.6 ±0.61	20	23.7 ±0.79	21	19.8 ±0.69	20	16.1 ±0.57	16	13.0 ±0.83
Lower abdominal	Transverse	12	32.6 ±0.96	10	27.8 ±0.62	16	17.8 ±0.62	20	13.8 ±0.72	17	11.2 ±0.48	11	9.5 ±0.55
Pulmonary artery	Transverse	4	33.8	1	29.0	5	27.9	9	21.1	8	24.6	5	19.3

* Variation in number of observations due to omission of measurements on damaged strips and because the entire aorta was sometimes not available.
** Mean % = amount of retraction divided by maximum stretched length × 100.
*** See text for exact localization of areas indicated in this column.

In order to correlate this selective loss of elasticity with the development of intimal lesions, the intimal weight of each portion is tabulated in Table II. In the descending thoracic portions where 2 strips were examined from each area, they were not averaged but included as independent determinations. This accounts for the greater number of observations in these areas. Other variations in the number of observations are due to the exclusion of damaged strips. The weight of the intima shows a progressive accretion with age although the increase is not so constant or so uniform as the decline in elasticity. For example, the mean intimal weight of the segments from the ascending aorta in the seventh decade is less than that for those in the sixth decade, although admittedly the number of observations in these particular groups are relatively few. The lower abdominal portion shows the first significant increase in weight over the other areas. Later the posterior thoracic and upper abdominal portions also develop heavier intimas than elsewhere. The ascending aorta and the anterior thoracic descending portions, as might be expected from casual observations, are covered by the lightest intimal layers. A positive correlation is apparent between the degree of loss of elasticity and the increase in intimal weight. Only in the anterior thoracic portion is there a marked discrepancy. This zone loses elasticity more rapidly in its longitudinal direction than do the transverse thoracic strips, yet its intima remains relatively thin.

The results are graphically recorded in Chart I where the degree of elasticity and the intimal weight are represented by equivalent intensities of shading. The selective loss of elasticity at different levels of the aorta with advancing age shows close coincidence with the increasing weight of the intima except for the one defection already noted.

Relation of Intimal Weight to Postmortem Elasticity of the Aorta

The thickened unyielding intima which develops with advancing age and increasing atheromatous deposits may serve to fix the underlying media and thus limit its flexibility to traction by mechanical interference. The alternative explanation is that the loss of elasticity in these selected areas is due to more rapid degenera-

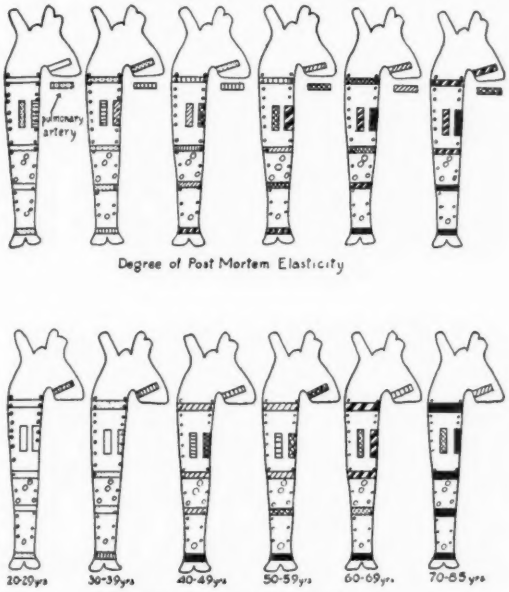
TABLE II

Relation of Intimal Weight to Age

Area	Direction	20–29 yrs.		30–39 yrs.		40–49 yrs.		50–59 yrs.		60–69 yrs.		70–85 yrs.	
		No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.	No.	Mean gm. per sq. cm.
Ascending	Transverse	9	0.036	9	0.044	10	0.044	12	0.068	10	0.045	12	0.065
Upper descending thoracic	Transverse	24	0.030	24	0.040	40	0.062	40	0.063	38	0.085	34	0.089
Lower descending thoracic	Transverse	23	0.026	23	0.039	40	0.061	40	0.065	40	0.077	31	0.102
Midthoracic descending anterior	Longitudinal	23	0.023	23	0.029	37	0.053	43	0.053	39	0.066	35	0.068
Midthoracic descending posterior	Longitudinal	20	0.031	23	0.039	36	0.066	44	0.071	35	0.087	33	0.115
Upper abdominal	Transverse	12	0.029	11	0.036	18	0.060	23	0.072	21	0.075	18	0.103
Lower abdominal	Transverse	10	0.038	6	0.049	16	0.088	21	0.088	18	0.089	11	0.140

tion of the elastic fibers, with weakening of the wall, and that the development of intimal plaques is a secondary phenomenon. In order to discover which of these two possible factors is responsi-

CHART I.—Comparison of Intimal Weight and Post Mortem Elasticity at Different Levels in Each Age Group.



ble, the following experiments were performed. In the thoracic areas the postmortem elasticity of the media alone, after the intima had been dissected away, was determined in a manner identical with the tests performed on immediately adjacent segments of intact aorta.

The results are shown in Table III, where the postmortem elasticity of the media alone denuded of its intima at various levels in

TABLE III

Influence of Intima on Postmortem Elasticity of Aorta at Various Ages

Area and direction	Layer tested	20–29 yrs.		30–39 yrs.		40–49 yrs.		50–59 yrs.		60–69 yrs.		70–85 yrs.	
		No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
Upper descending thoracic	Media * only	13	37.8 ±0.54	12	35.4 ±0.42	20	31.4 ±0.34	21	27.9 ±0.55	21	23.7 ±0.48	17	19.0 ±0.61
Transverse	Intact	15	37.0 ±0.46	13	33.7 ±0.52	23	29.7 ±0.42	21	27.0 ±0.58	21	21.5 ±0.48	18	16.2 ±0.63
Lower descending thoracic	Media only	11	39.0 ±0.50	12	36.5 ±0.45	19	31.8 ±0.24	22	28.8 ±0.61	20	24.5 ±0.45	16	18.9 ±0.63
Transverse	Intact	14	38.2 ±0.46	12	34.3 ±0.63	22	29.8 ±0.38	21	26.6 ±0.71	20	21.1 ±0.48	16	16.1 ±0.54
Midthoracic descending anterior	Media only	9	34.9 ±0.67	11	28.9 ±0.63	16	25.6 ±0.53	22	25.2 ±0.51	19	21.5 ±0.56	17	18.7 ±0.63
Longitudinal	Intact	12	33.5 ±0.51	12	28.5 ±0.51	22	22.9 ±0.46	22	21.4 ±0.54	20	17.2 ±0.53	17	14.2 ±0.43
Midthoracic descending posterior	Media only	9	33.0 ±0.72	11	28.3 ±1.04	17	24.4 ±0.60	21	21.9 ±0.60	15	21.0 ±0.60	17	17.6 ±0.61
Longitudinal	Intact	11	30.3 ±0.75	12	26.8 ±0.52	21	19.6 ±0.41	22	17.6 ±0.53	20	12.7 ±0.53	17	11.2 ±0.49

* Intima removed from strips by dissection before testing.

the thoracic portion is compared in different age groups with samples of the intact aorta at the same levels. It may be seen that the presence of the intima invariably reduces the degree of retraction of the intact aorta under the conditions of the experiment. This difference is relatively slight in the early decades of life but becomes more marked in older age groups and particularly so in those areas that develop the heaviest intimas. For example, at the posterior thoracic portion in the two oldest age groups the freed media exhibits an elasticity that is 50 to 60 per cent greater than the intact segment. Nevertheless, it is also apparent that the media itself progressively loses its power to retract in older age groups. The reduction in the case of the stripped media is even more uniform throughout life than it is for the intact strip. It is greater in the longitudinal than in the transverse plane. The anterior longitudinal portion shows as rapid a decline as does the posterior longitudinal, so that the greater losses of the latter in the intact condition are largely due to the restraint offered by the thicker accumulations of intimal atheromas which so frequently develop at this site.

The above experiments indicate that the loss of postmortem elasticity which occurs with advancing age is due largely to a failure on the part of elastic fibers to recoil as efficiently after extension, and it is also due in part to the resistance offered by the thickened intima. The possibility still exists that the media, if held immobile beneath intimal plaques, may secondarily lose its ability to stretch and retract perhaps because of disuse. There is some indication that the greatest losses of elasticity occur at points where the vessel is most rigidly attached *in situ,* namely, along the posterior wall and at the bifurcation.

In order to examine more closely for a relation between postmortem elasticity and intimal thickening, the changes within each age group were explored. In Table IV the segments within each decade are grouped by intimal weight and the elasticity of those covered by the thickest intimas compared with those covered by the thinnest ones. When this is done the number of observations falling into each division are so few that some values show chance variation. This is particularly true when only one observation is recorded. Nevertheless, it may be seen that there is a striking tendency for the mean values of each group within a

TABLE IV

Relation of Postmortem Elasticity to Intimal Weight of Intact Aorta Within Each Age Group

Age in yrs.	Intimal weight (gm. per sq. cm.)	Ascending aorta		Upper descending thoracic transverse		Lower descending thoracic transverse		Midthoracic descending anterior longitudinal		Midthoracic descending posterior longitudinal		Upper abdominal transverse		Lower abdominal transverse	
		No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
20–29	0.010–0.019	1	40.0	1	38.8	4	39.8	3	35.1	1	30.4	5	35.7	1	32.7
	0.020–0.029	3	39.4	9	37.2	2	34.0	5	32.5	6	32.8	1	39.5	3	35.2
	0.030–0.039	3	35.4	4	37.0	3	38.7	2	31.9	1	32.0	2	37.8	3	31.6
	over 0.040	1	35.7	1	33.9	3	38.1	3	33.0	2	34.7	4	36.2	3	32.5
30–39	0.015–0.029	1	32.8	2	34.7	1	35.3	5	28.2	1	21.0	3	33.0	1	27.1
	0.030–0.039	1	35.4	7	34.3	7	33.6	3	28.9	5	27.0	4	33.9	2	30.7
	0.040–0.049	4	35.6	2	31.8	2	34.6	3	28.2	6	28.3	4	29.6	1	29.6
	over 0.050	3	31.8	2	34.5	2	36.0	1	30.4	1	27.6	.	..	3	26.2
40–49	0.019–0.039	6	29.7	6	30.9	6	29.0	8	23.6	3	20.6	4	25.4	2	19.4
	0.040–0.059	2	30.6	8	31.3	12	31.2	11	22.1	11	20.0	6	27.6	3	18.8
	0.060–0.079	1	29.2	6	28.6	1	29.9	1	23.2	4	21.1	2	24.0	1	16.7
	0.080–0.099	.	..	.	..	2	26.8	.	..	1	13.8	3	22.6	4	16.8
	over 0.100	1	32.2	2	26.6	2	27.7	2	23.8	2	16.0	4	18.5	6	17.6
50–59	0.020–0.039	1	16.8	5	29.3	3	26.1	8	22.0	1	20.0	3	23.8	4	16.6
	0.040–0.059	7	27.9	6	24.3	8	26.4	8	21.9	6	17.6	10	20.9	4	18.9
	0.060–0.079	1	29.2	7	27.9	8	27.7	5	19.6	8	19.2	2	21.0	3	10.4
	0.080–0.099	1	25.0	2	24.6	.	..	2	21.2	5	16.2	2	15.6	1	13.8
	over 0.100	2	20.0	1	19.1	2	24.7	.	..	2	13.2	4	15.1	9	11.3
60–69	0.020–0.039	3	22.9	1	28.2	2	24.6	5	18.3	.	..	2	16.3	4	12.8
	0.040–0.059	4	24.6	4	20.6	4	19.4	3	20.2	3	18.1	7	17.9	2	12.5
	0.060–0.079	2	24.1	6	22.1	7	22.2	5	16.1	4	13.8	3	15.4	4	9.4
	0.080–0.099	.	..	5	21.9	4	19.7	3	17.0	6	11.6	3	17.4	1	13.5
	over 0.100	.	..	4	19.0	4	20.7	2	12.6	7	11.3	4	12.6	5	9.5
70–85	0.030–0.059	5	17.4	4	20.1	2	16.0	10	14.6	1	11.1	2	15.7	2	13.5
	0.060–0.079	3	16.5	7	15.5	5	16.5	4	15.1	1	14.8	6	13.2	.	..
	0.080–0.099	2	15.5	2	15.4	5	16.4	2	11.6	4	10.3	1	25.3	2	9.8
	0.100–0.119	.	..	4	14.9	2	14.5	2	13.8	3	11.5	3	11.5	1	10.5
	over 0.120	1	8.0	1	12.1	2	10.4	.	..	2	9.2	4	9.8	5	7.4

given decade to approximate each other. In the groups with heavier intimas there is a slightly lessened degree of elasticity. This is especially the case when the average intimal weight exceeds

TABLE V

Relation of Postmortem Elasticity of Media Denuded of Intima to Intimal Weight Within Each Age Group

Age in yrs.	Weight of intima (gm. per sq. cm.)	Upper descending thoracic transverse		Lower descending thoracic transverse		Midthoracic descending anterior longitudinal		Midthoracic descending posterior longitudinal	
		No.	Mean %	No.	Mean %	No.	Mean %	No.	Mean %
20–29	0.010–0.019	.	..	4	30.5	4	34.1	1	30.4
	0.020–0.029	7	38.9	3	39.0	3	33.6	6	32.8
	0.030–0.039	1	36.8	2	38.9	1	35.2	1	32.0
	over 0.040	3	36.8	1	36.7	1	36.8	2	34.7
30–39	0.015–0.029	2	37.0	3	37.7	4	28.5	2	26.0
	0.030–0.039	3	36.5	3	37.0	2	33.3	6	29.3
	0.040–0.049	2	35.8	2	36.9	4	29.0	1	31.1
	over 0.050	4	34.0	2	36.8	.	..	2	25.3
40–49	0.019–0.039	4	30.4	2	30.7	5	26.5	.	..
	0.040–0.059	5	32.9	6	32.8	6	25.2	5	24.3
	0.060–0.079	4	32.5	7	31.4	3	27.0	4	25.3
	0.080–0.099	5	29.2	1	31.7	1	22.2	3	23.3
	over 0.100	1	32.9	2	31.7	1	28.2	2	25.9
50–59	0.020–0.039	4	26.8	1	32.9	3	22.0	1	17.3
	0.040–0.059	5	29.8	8	29.0	10	24.0	8	20.5
	0.060–0.079	5	27.2	5	26.9	3	28.2	5	22.9
	0.080–0.099	2	28.8	2	26.2	2	23.4	2	24.9
	over 0.100	2	26.9	3	29.4	1	30.4	4	23.2
60–69	0.020–0.039	1	20.4	.	..	3	22.6	2	22.2
	0.040–0.059	3	25.2	3	23.9	4	24.3	1	21.2
	0.060–0.079	5	23.8	7	25.5	4	21.8	3	21.6
	0.080–0.099	5	25.2	5	23.3	4	19.2	4	23.4
	over 0.100	5	23.8	2	25.1	3	21.0	3	20.3
70–85	0.030–0.059	2	25.3	.	..	3	21.9	.	..
	0.060–0.079	4	17.5	4	19.3	7	16.8	1	17.3
	0.080–0.099	4	21.3	1	15.3	3	19.6	7	18.6
	0.100–0.119	4	15.6	4	17.4	.	..	3	14.4
	over 0.120	3	17.9	5	20.5	2	20.4	5	17.0

0.100 gm. per sq. cm. As previously noted, at this weight macroscopically appreciable calcification is usually present. It may be concluded then that within a single age group the degree of postmortem elasticity of the intact aorta declines slightly as the intimal

plaques become more extensive. The variation with intimal weight is minimal when compared with the loss of elasticity that occurs with advancing age so that there is very little overlapping between the various age groups.

When the postmortem elasticity of the media denuded of its intima is examined within each age group with reference to the weight of the detached intima in the same manner, a different result is obtained as shown in Table V. Here the postmortem elasticity of the stripped media is found to be fairly constant at the same level for each age period regardless of the weight of the intima. Such variations as do occur are probably the result of paucity of data, but obviously the media of the segment bearing the heaviest intimas is as elastic as those covered by the thinnest ones in most instances. This would indicate that the decline in elasticity of the intact aorta associated with increasing intimal weight within a single age period, as shown in Table IV, is simply due to the resistance offered by the unyielding intima which becomes more effective as the membrane thickens.

Discussion

From this elementary approach to the problem a number of significant observations are evident. First of all, the experience of many previous workers is substantiated, namely that there is a progressive reduction in the extensile and retractile properties of the aorta which continues throughout adult life. This reduction develops at a quite uniform rate for a given area and appears to affect all persons to approximately the same extent. The postmortem elasticity is therefore almost entirely dependent on age. Indeed, the uniformity within a single age group is quite striking, indicating that the elasticity is not readily altered either by disease processes or postmortem changes.

The loss of elasticity, although constant for a given area, varies in different portions. It is more rapid and complete in the longitudinal direction than transversely, along the posterior aspect more than anteriorly, and at the bifurcation rather than at the proximal portions. The only factor that appears to be constant for all regions in which the reduction in elasticity occurs earlier and more rapidly is that they represent portions which by reason of

their attachments during life must have had a limited excursion with each pulse beat. Obviously, a living artery must expand more transversely with each pulsation than longitudinally. The posterior wall and the bifurcation represent areas that are again more firmly united to adjacent bony structures and thus of limited mobility. This suggests either that fixed areas of the aorta lose elasticity simply through relative disuse, or that regenerative or replacement processes are so directed as to maintain the more mobile parts of the aorta in a more elastic condition. Another possibility is that the more firmly attached portions are subject to greater stress and strain. This, however, does not seem very likely and would be difficult to prove or disprove.

There does not appear to be any very direct association between the degree of postmortem elasticity and the extent of intimal lesions. Reduction in elasticity of the media proceeds as rapidly and regularly in those individuals who develop a few small atheromas as in those who develop many large ones. It is true that in the preceding experiments a thickened intima sometimes retarded the mobility of the media but under the lesser degrees of tension to which the aorta is subjected during life it is doubtful whether this degree of limitation is sufficient to hamper the necessary movements of the vessel. The observation that the pulmonary artery undergoes a similar diminution in postmortem elasticity with increasing age, albeit to a somewhat lesser degree, also would seem to divorce this change from an essential relation to the formation of intimal plaques. As is well recognized, this vessel is almost never the seat of intimal lesions unless some disease process interferes seriously with the pulmonary circulation.

The curious localization of the early atheromatous lesions in the areas subject to the greatest losses of elasticity may be entirely unrelated, although it does suggest an indirect but not very obvious connection. If the assumption is made that lipoid material, once it has gained entrance into the intima, is capable of being shifted in position, then a plausible explanation can be offered. There might well be a tendency for it to gravitate to the least elastic and movable parts. A number of attendant circumstances make it seem likely that such an event may transpire. First of all, the earliest microscopically visible accumulations of lipoid material are contained within phagocytes capable of movement. Secondly, there

is a tendency for lipoid material to collect about intimal scars, such as the point of closure of the ductus Botalli, at the commissural margins of the aortic valve cusps, or about the orifices of the intercostal arteries. This may be interpreted as evidence that the lipoid material has penetrated along tissue spaces in the intima until it reached an impassable barrier.

The usual explanation offered for the focal distribution of intimal lipoid deposits is that they occur at points of great stress and strain. There is little positive evidence to support this contention. If anything, one might suspect that the posterior portion of the thoracic aorta would be more protected from strain by reason of its attachment to bone. Similarly, one would also expect that the anterior, more movable regions, where the large abdominal vessels arise, would be subjected to greater stress and strain, yet these areas are less often involved by early lipoid deposits. Another more striking example is afforded by the mitral valve leaflets. Here obviously the greatest point of stress and strain must be at the contact or distal edges of the leaflets. Yet lipoid deposits accumulate not infrequently only at the basal, less movable and more protected portions. The ease with which the intima may be detached from the media after death is an indication that these two layers are but loosely amplectant and suggests planes of dissection along which lipoid may be transferred. If lipoid material within the intima is capable of spreading, an attractive explanation for the development of focal accumulations is apparent. The lipoid may well gain entrance initially in minute amounts over large areas of the surface and only later come to lodge in discrete circumscribed deposits.

The reduction in elasticity with age is probably chiefly dependent on changes in the elastic fibers. Neither the smooth muscle nor the connective tissue can play a very fundamental role, both by reason of the physical properties of each of these two tissues and because of their architectural arrangements within the media. Inert smooth muscle strips, as represented by the muscularis of the intestine, will, it is true, undergo marked elongation when subject to traction but will fail to retract on release to more than 10 per cent of the maximum stretched length. Connective tissue, as exemplified by chordae tendineae and tendons, offers great resistance to traction and can only be stretched to a length about

6 per cent greater than when released. Inasmuch as even in senile persons the aorta still possesses as much retractility as either of these two types of tissue, it seems fairly reasonable to assume that any greater degree of retraction is dependent on the properties of the elastic fibers. Architecturally, the framework of the smooth muscle is anchored to the delicate reticulum sheaths that enclose the elastic fibers. The effects of traction depend therefore on the ability of the elastic fibers to elongate.

The most striking alteration that the elastic fibers undergo with advancing age is a loss of undulation. Loss of elasticity as well as dilatation of the vessel as a whole is probably reflected by this failure on the part of the elastic fibers to recoil on relaxation as efficiently as formerly. Ranke [20] has shown that the elastic fibers have properties analogous to fine metal coils and that stretching is accomplished merely by the straightening of the coils under tension and not by any yielding or thinning of the substance of the fiber itself. In vessels distended and fixed in formalin at pressures exceeding the greatest ever experienced during life, the fibers are almost but not quite straight, whereas with decreasing pressures the wavy contours return. An opposite point of view has been maintained, most recently by Andrus,[21] that the elastic fibers are rubbery in nature and stretching is accomplished by their thinning out. The degree of undulation is from this point of view merely slack caused by excessive muscular contraction and true stretching does not begin until the elastic fibers are straight.

Some of the confusion over this relatively simple point may have been caused by the fact that 10 per cent formalin fixation for 24 hours does not destroy completely, although it greatly reduces, the elastic properties of elastic tissue. Arteries fixed in a distended state in this manner may show some retraction when freed from pressure that might alter the histological appearance. Support for Ranke's concept can be easily obtained by stretching 1 of 2 adjacent strips of aorta with a weight of 1500 gm., attaching the extended strip to a firm support to prevent retraction and fixing both the stretched and relaxed strips in Zenker's fluid. This fixative completely abolishes the elastic properties of the vessel wall. As seen in the microphotographs, taken at the same magnification, of sections prepared with Weigert's elastic tissue stain, maximum stretching is accomplished without any thinning of the

elastic fibers which remain the same in diameter as in the undistended strip. After death at least, the elasticity of elastic fibers is not comparable to that of fine rubber bands.

Since a fine metal coil resists compression as well as extension and has a tendency to return to a fixed shape when relaxed, it is easy to understand why an aorta freed of its *in situ* attachments tends to assume a constant shape and size dependent on the ability of the elastic fibers to recoil. This force is presumably great enough to overcome any resistance on the part of the smooth muscle and connective tissue.

Summary and Conclusions

The postmortem elasticity of the aorta as measured by the ability of excised portions to retract following extension is a constant and durable property which varies only with age. In the young adult the elasticity is approximately equal in all areas and in both the transverse and longitudinal directions. With advancing age there is progressive loss of elasticity which varies in degree in different areas but proceeds at a constant rate for a given area and affects all persons to approximately the same extent. This reduction in elasticity is associated with a failure on the part of the individual elastic fibers to recoil and assume a wavy appearance on relaxation and is commensurate with the enlargement of the vessel as a whole.

Reduction in elasticity occurs first and proceeds more rapidly in those areas that are most rigidly attached *in situ* and in the longitudinal plane. Such areas are presumably capable of less movement with each pulsation during life.

The development of intimal plaques is not directly related to this loss of elasticity. A greatly thickened intima can restrict the elasticity of the media to some extent. Loss of elasticity of the media proceeds as rapidly in those subjects who develop slight intimal atheromas as in those who exhibit profound changes. The earliest and largest intimal accumulations of lipoid material appear in those areas that are subject to the earliest and most marked loss of elasticity. An hypothesis is advanced to account for this coincidence. This is based on the assumption that lipoid material does not necessarily remain at its point of entrance into the intima

but may be influenced by the movements of the vessel during life to lodge in the least elastic and least movable portions. Loss of elasticity with age occurs constantly in an artery, the pulmonary one, not usually the seat of intimal plaques.

REFERENCES

1. Polotebnow. Sklerose des arteriellen Systems als Ursache consecutiver Herzerkrankungen. *Berl. klin. Wchnschr.*, 1868, **5**, 361–363.
2. Wertheim, M. G. Memoire sur l'elasticité et la cohesion des principaux tissus du corps humain. *Ann. de Chemie et de Physique*, 1847, Series III, 385.
3. Wundt, Wilhelm. Die Lehre von der Muskelbewegung. F. Viewig und Sohn, Braunschweig, 1858.
4. Kaufmann, L. Zur Frage der Aorta Angusta: Ein Beitrag zu den Normalmaassen des Aortensystems. Veröff. aus dem Gebiete der Kriegs- und Konstitutions-Pathologie. Gustav Fischer, Jena, 1919, **2**.
5. Wells, H. G. The chemistry of arteriosclerosis. Arteriosclerosis: A Survey of the Problem, Cowdry, Edmund V. The Macmillan Company, New York, 1933, Chapt. 11.
6. Aschoff, L. Introduction. Arteriosclerosis: A Survey of the Problem, Cowdry, Edmund V. The Macmillan Company, New York, 1933.
7. Reuterwall, Olle P. Über die Elasticität der Gefässwände und die Methoden ihrer näheren Prüfung. *Acta med. Scandinav., Suppl. 2*, 1921, **4**, 1–175.
8. Yater, Wallace M., and Birkeland, Ivar W. Elasticity (extensibility) of the aorta of human beings. *Am. Heart J.*, 1929–30, **5**, 781–786.
9. Roy, Charles S. The elastic properties of the arterial wall. *J. Physiol.*, 1880–82, **3**, 125–159.
10. Herringham, W. P., and Wills, W. A. On the elasticity of the aorta; being a contribution to the study of arterial sclerosis. *Medico-Chir. Tr.*, 1904, **87**, 489–530.
11. Israel, O. Ueber erworbene Störungen in den Elasticitätsverhältnissen der grossen Gefässe. *Virchows Arch. f. path. Anat.*, 1886, **103**, 461–473.
12. Suter, F. Ueber das Verhalten des Aortenumfanges unter physiologischen und pathologischen Bedingungen. *Arch. f. exper. Path. u. Pharmakol.*, 1879, **39**, 289–332.
13. Hiller, R. Ueber die Elasticität der Aorta. Diss. Halle a. S., 1884.
14. Faber, Arne. Die Arteriosklerose; ihre pathologische Anatomie, ihre Pathogenese und Ätiologie. Gustav Fischer, Jena, 1912.
15. Thoma, R., and Kaefer, N. Ueber die Elasticität gesunder und kranker Arterien. *Virchows Arch. f. path. Anat.*, 1889, **116**, 1–27.

16. Bramwell, C. Physical properties of arteries in health and disease. Arteriosclerosis: A Survey of the Problem, Cowdry, Edmund V. The Macmillan Company, New York, 1933, Chapt. 3.

17. Aschoff, Ludwig. Atherosclerosis. Lectures on Pathology. Paul B. Hoeber, Inc., New York, 1924, Chapt. 6.

18. Triepel, Hermann. Einführung in die physikalische Anatomie. J. F. Bergmann, Wiesbaden, 1902.

19. Krafka, Joseph. The mechanical factors in arteriosclerosis. *Arch. Path.*, 1937, **23**, 1–19.

20. Ranke, Otto. Zur Frage der elastische Systeme, besonders der Aortenwand. *Beitr. z. path. Anat. u. allg. Pathol.*, 1925, **73**, 638–656.

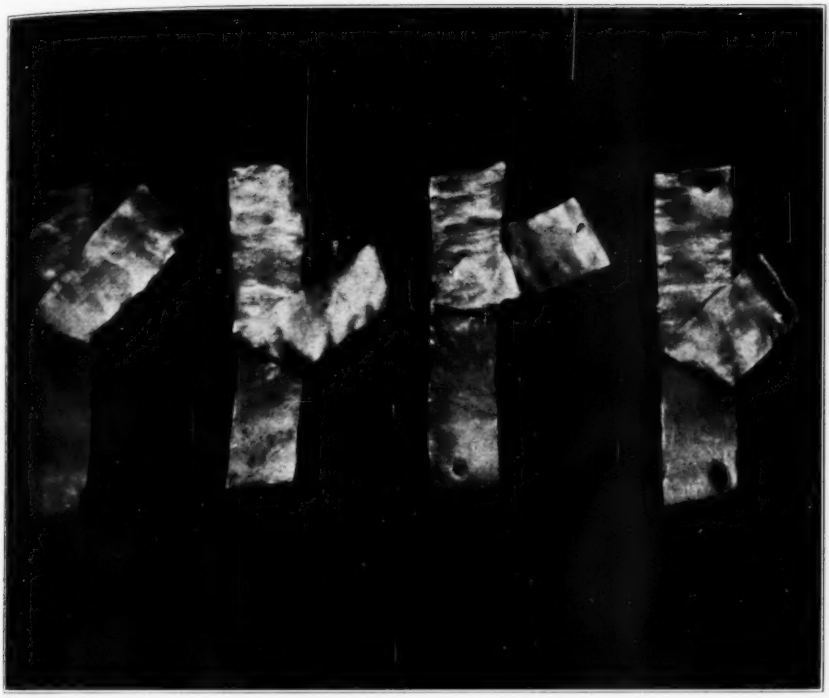
21. Andrus, Frank C. The relation of age and hypertension to the structure of the small arteries and arterioles in skeletal muscle. *Am. J. Path.*, 1936, **12**, 635–654.

DESCRIPTION OF PLATES

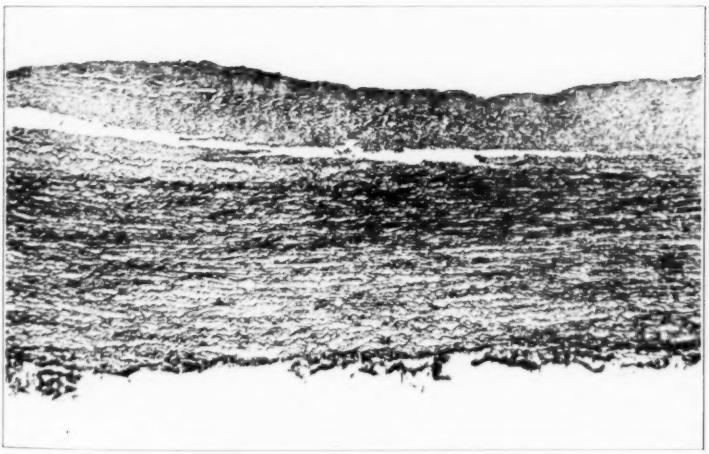
Plate 112

Fig. 1. Strips of aorta with the intimal layers partly freed and reflected backward. The intima separates as a thin membrane including the bulk of intimal plaques. The exposed medial surface is smooth and intact.

Fig. 2. Microphotograph of strip of aorta with intima detached from media. The clear space represents the line of separation which lies deep within the intima and leaves the underlying media relatively undamaged. Weigert's elastic tissue stain. × 50.



1

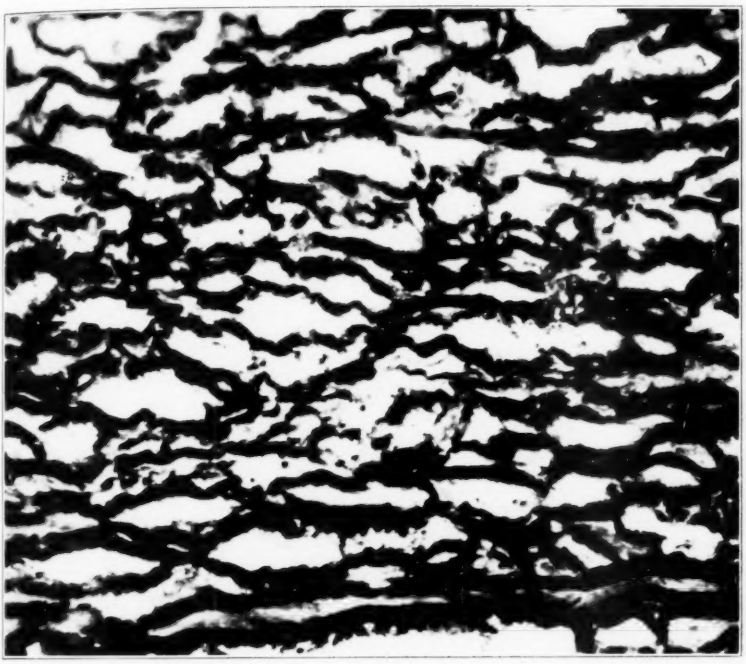


2

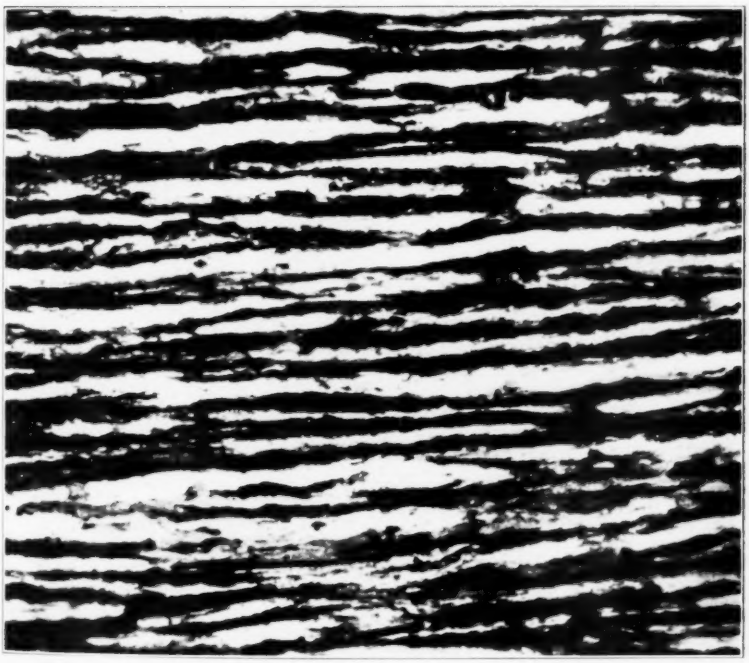
UNIV. OF MICH.

PLATE 113

FIGS. 3 and 4. Microphotographs taken at same magnification of two adjacent strips of aorta. The upper one was fixed in the usual manner; the lower one while stretched with 1500 gm. of traction. In the unextended strip the elastic lamellae show their usual wavy contours. In the stretched one they have become straightened, lie closely together and are parallel to each other, but the average thickness of the fibers is unchanged. Weigert's elastic tissue stain. × 300.



3



4

UNIV. OF MICH.

LOCALIZED CONGENITAL DEFECTS OF THE CARDIAC INTERVENTRICULAR SEPTUM *

A Study of Three Cases

David G. Mason, M.D., and Warren C. Hunter, M.D.

(From the Department of Pathology, University of Oregon Medical School, Portland, Ore.)

Phylogenetically and pathologically cardiac malformations have long excited a lively interest but only comparatively recently have cardiologists given serious consideration to the matter of establishing as certainly as possible the nature of such conditions during life. That accuracy of diagnosis in congenital heart disease can be improved is shown by the figures of McGinn,[1] who reports that by means of careful and systematic study the incidence of correct or suggested diagnoses was raised from a previous percentage of 48 to 85 per cent. As evidence that pathologists are becoming increasingly alert in searching for cardiac anomalies, McGinn also points out that among 4100 autopsies falling in the period between 1895 and 1920 congenital heart lesions had an incidence of 0.6 per cent as compared with 1.2 per cent among 3400 cases examined during the 1921–1935 interval, an increase felt to be the direct result of a greater interest in the growing accumulation of information about malformations of the heart.

Among the rarer developmental abnormalities of the heart are localized defects of the interventricular septum, not at the base of the heart just anterior to the pars membranacea, which is the common site, but elsewhere in the septum or multiple. In only 5 of the 1000 cases analyzed by Abbott [2] is this condition listed as primary, and it occurred as a complication of other anomalies in only twelve additional hearts. By way of contrast it may be mentioned that the same statistics show 257 cases of defects at the pars membranacea. From the standpoint of comparative anatomy Abbott and Shanly [3] have stated that the homologue of septal

* Received for publication May 11, 1937.

defects of the type under consideration exists in the python (*P. murulus*), in which there exists normally a communication low in the septum between the dorsal and ventral ventricles.

In the small group of muscular septal imperfections the case described by Weiss [4] stands in a class by itself. His subject was a male who attained the mature age of 79 years without cardiac symptoms, aside from a purely terminal cyanosis. Auscultation revealed a loud, harsh, systolic murmur, most intense over the ensiform but still audible over the entire cardia; the aortic and pulmonic sounds were not accentuated. The heart borders were not easily defined but did not seem to be increased. Edema was absent. The electrocardiogram displayed a rate of 84, normal atrioventricular bundle conduction, impaired and delayed right branch bundle conduction, and evidence of right ventricular preponderance and of myocardial degeneration. The unusual position of the intense thrill and the harsh quality of the murmur, together with the peculiar cyanosis, suggested the possibility of a latent congenital lesion such as a septal defect, and this was the probable clinical diagnosis although the location of the communication could not be established.

Postmortem examination of the heart disclosed a circular defect, 2 cm. in diameter, located in the posterior half of the interventricular septum about midway between the apex of the left ventricle and the mitral ring and communicating with the right ventricle near the apex by means of an aneurysmal pouch and four or five fenestrations. The edge of the hole on the left side of the septum was fibrous and smooth but directly above it was a roughened spot covered by a mural thrombus. Neither of the ventricular walls was thicker than normal. The features distinguishing the lesion as congenital were the location, size and smooth edges of the defect, together with the natural openings between the trabeculae carneae on the right ventricular aspect.

Among nearly 8000 autopsies on individuals of all ages we have encountered congenital imperfections in the muscular division of the interventricular septum three times, always in children of 12 years or less. In two of these the only important anomaly is a single point of communication between the ventricles. One of these we believe to be unparalleled in the literature for the reason that while the muscle is interrupted the actual opening is so

small that little if any shunting of blood could have taken place. In the third heart there are two points of interventricular communication in an otherwise essentially normally developed organ.

Case Reports

Case 1: (No. 9546). No information concerning this 12 year old white boy is available other than that he dropped dead while attending church services. The autopsy was ordered by the coroner.

Description of Heart

Aside from the inconsequential congenital fusion of two costal cartilages, the only congenital condition is in the heart, which is dilated and pushes the left lung toward the thoracic wall. The heart and aorta as far as the midthoracic level weigh 165 gm. The organ is rotated so that the anterior presenting surface is formed by the right chambers alone. The right atrium in particular is dilated. The foramen ovale is closed; the thebesian valve persists. There exists a communication (Fig. 1) between the ventricles through the muscular part of the septum immediately adjoining the junction of the septum with the anterior wall of the heart. The ostium measures 1 by 0.8 cm. on the left ventricular side, 1 by 0.6 cm. on the right side, and courses obliquely caudalward from left to right. The cephalad margin of the opening lies 1.5 cm. below the junction of the muscular and the membranous parts of the septum. The latter is quite intact. Along the anterior margin of the defect are two ridge-like trabeculae carneae; elsewhere the border is smooth and even. The left and right ventricular endocardium fully covers the defect and while thicker and more whitish than elsewhere is free from any signs of vegetations or thrombi. The ventricles appear to be of equal size, although by actual measurement the wall of the right proves to be 0.8 cm. in thickness at the apex and 1.3 cm. at the base, as compared to 0.7 and 1.1 cm. for the left at the same points. The posterior and left anterior leaflets of the pulmonary valve are fenestrated, as are also the anterior and left posterior cusps of the aortic valve, although to a lesser degree. There are three right coronary ostia, measuring 3, 1.5, and less than 0.5 mm., respectively. The left coronary artery ostium has a diameter of 3.5 mm. The

distribution of the coronary vessels is the most frequent of the normal varieties. The tricuspid and mitral valves are unchanged save for three oval-shaped atheromatous plaques in the anterior leaflet of the latter valve. Several transversely directed fatty streaks occur in the aortic intima at the level of the valve commissures. The main pulmonary artery is similarly involved, except that the plaques extend onward into the left primary branch. The pulmonary valve has a circumference of 6.3 cm., while the aortic ring measures 4.5 cm. The great vessels enter and leave the heart in the normal manner. The ductus arteriosus, although patent, is only 1 mm. in diameter.

Anatomical Diagnoses: Congenital malformations of the heart: (*a*) isolated defect of muscular portion of interventricular septum, (*b*) fenestrated aortic and pulmonic valve cusps, and (*c*) multiple (3) right coronary ostia; persistent patent ductus arteriosus; persistent thebesian valve; hypertrophy of right ventricle; pronounced dilatation of right atrium; moderate dilatation of left ventricle; atheroma of anterior mitral valve leaflet, intima of ascending aorta, main pulmonary artery and its left branch; generalized acute passive hyperemia; and congenital fusion of two costal cartilages.

Alteration of the Circulation: The venous blood entering the right side of the heart was propelled by the hypertrophied right ventricle into the pulmonary bed in the usual way. Some oxygenated blood from the left ventricle no doubt was ejected into the right ventricle through the imperfect septum during systole providing the normally higher left ventricular pressure existed. In support of this concept is the preponderance of the pulmonary valve ring over the aortic and the greater thickness of the right ventricular wall. While the ductus arteriosus was patent it appears to be too small to have had any functional significance.

CASE 2: (No. 538–10–35): The subject was a boy, 6 years of age, with an abnormally long bleeding and clotting time who died from hemorrhage following tonsillectomy and adenoidectomy. There is no history of any cardiac symptoms during life, the heart lesion being discovered in the course of a routine autopsy.

Description of Heart

The right side of the heart is much dilated and is largely responsible for the transverse diameter of 13 cm. The whole organ

weighs 110 gm. It is normally formed save for a minute fenestration of the right posterior aortic valve leaflet, the presence of three right coronary artery ostia, two of which are very small, and the interruption of the muscle of the interventricular septum. On viewing the septal aspect of the left ventricle one's attention is drawn to a pit-like area surrounded by a rim of pearly white endocardium (Fig. 2), situated about midway between the apex and the aortic ring, 5 mm. from the junction of the septum with the anterior wall and 9 mm. from the posterior wall. The trabeculae carneae on the septal side end abruptly at the inferior margin of the lesion. The raised whitish rim about the crater-like depression measures 6 by 4 mm. A sagittal cut carried directly through the apparent stoma reveals a complete interruption of the muscle of the septum at this level by what appears at first glance to be a solid cord of whitish tissue joining with the endocardium of either ventricle. However, on more careful inspection there is discovered at the center of the apparent cord a minute opening 0.5 to 1 mm. in diameter having a smooth and glistening lining but devoid of any blood. The stoma of the defect on the right ventricular side is slit-like and much less prominent than that on the left side. The exact point of attachment of the thickened band of endocardium projecting outward into the right ventricle (Fig. 2) is unknown, owing to the fact that it was divided in opening the right ventricle at autopsy before the presence of the abnormality was discovered. When the cut margins are reapproximated, however, the band comes to lie very near the base of the papillary muscle of the anterior tricuspid valve leaflet. The right terminus of the defect is 5 mm. from the point where the anterior wall of the right ventricle joins with the interventricular septum. On the left side of the septum the interrupted myocardium is rounded off along either side of the fibrous endocardial tube, but on the right side only the inferior border is bevelled. Two yellowish atheromatous patches occur in the anterior mitral leaflet and a number of similar streaks involve the aortic intima within the sinuses of Valsalva and just superior to them. Both the foramen ovale and the ductus arteriosus are obliterated.

Anatomical Diagnoses: Congenital malformations of the heart: (*a*) defect of septum interventriculorum through its muscular portion, (*b*) fenestrated right posterior aortic valve leaflet, and (*c*)

multiple (3) right coronary ostia; focal fibrosis of endocardium in immediate vicinity of defect; atheroma of aorta and anterior mitral valve cusp; and acute dilatation of the right heart chambers.

Alteration of the Circulation: Neither the clinical history, physical examination nor the pathological observations indicate that any significant amount of shunting of blood took place through this minute anomalous communication. The pronounced dilatation of the right heart was acute and can be accounted for by the fatal postoperative hemorrhage.

Case 3: (No. 207–4–32). This 7 year old girl collapsed and died suddenly after being struck over the manubrium sterni by a batted indoor baseball. She was roller skating at the time of the accident. Dr. Roy A. Payne, a neighbor of the family, had been asked rather casually to examine her about a year previously, and finding some enlargement of the heart to the left, together with a double murmur over the mitral area, requested the parents to bring the child to his office for a complete study, but this was not done and he did not see the girl professionally again. He had noted that she was able to play like other children of her age but seemed to fatigue easily.

Description of Heart

At autopsy there was found a zone of hemorrhage in the soft tissues over the manubrium sterni at the point of impact of the ball. All organs except the heart were normally developed.

The heart weighs 150 gm. Anteriorly the right ventricular wall has a thickness of 1 cm. and at the base posteriorly measures 1.4 cm., as compared to 1 cm. and 1.3 cm. for the left ventricle at corresponding points. The myocardium displays no macroscopic changes. The tricuspid valve has a circumference of 9.5 cm., including a point of cleavage 1.6 cm. in extent situated between the inferior and medial leaflets. Otherwise this valve is unchanged. The membranous part of the interventricular septum is lacking and in its place is an oval opening (Fig. 3) 1.8 by 2.3 cm. in size with the longer diameter directed somewhat anteroposteriorly. The superior border of the hole is formed by the smooth and sharp edged interatrial septum with parts of the inferior and medial cusp of the tricuspid valve forming the upper anterior and posterior margins. All other parts of the boundary consist of the septal myocardium, covered by normal appearing endocardium. Where the muscular part of the interventricular septum joins the posterior wall of the heart is a second defect situated between two large trabeculae carneae. On the right side of the

septum the opening measures 0.7 by 1.4 cm., the long diameter being directed superior-inferiorly and the lesser one anteroposteriorly. On the left ventricular aspect the opening is smaller, measuring 0.6 by 0.8 cm. The endocardium covering the perforation in unchanged. Crossing the inferior border on the left side is a large bundle of muscle, obviously one of the trabeculae carneae, which passes cephalad to end at the inferior border of the defect at the site of the absent membranous septum. Posterior to this band and at the same level as the opening in the muscular division of the partition is a third fenestration 2 by 2 mm. in size which communicates with the main opening. The mitral valve circumference is 8 cm. The posterior leaflet is shorter than its mate and possesses two distinct papillary muscles, one quite small, the other of average normal size. The anterior cusp has a thick and rolled margin, and like the other leaflet has two very stout papillary muscles. All chordae tendineae attached to this cusp are short, thick and pearly white. No verrucae or other forms of vegetations occur on either of the mitral cusps. The anterior mitral leaflet is on a level with the opening through the membranous septum and prevents the latter from being seen from the left ventricular side. Viewed from the right ventricle the mitral leaflet forms a curtain-like background along the upper half of the defect. The myocardium is everywhere unaltered. The aortic valve ring has a circumference of 3.2 cm., the pulmonic a circumference of 5.5 cm. In the intima of the ascending aorta and the main pulmonary artery are a few fatty streaks. The coronary arteries are normally distributed. The foramen ovale and ductus arteriosus are closed. There are no abnormalities of development or of position on the part of any of the great vessels leaving and entering the heart.

Anatomical Diagnoses: Multiple congenital lesions of the heart: (*a*) defective interventricular septum — (1) pars membranacea and (2) pars muscularis, (*b*) cleavage of inferior and medial leaflets of the tricuspid valve, (*c*) supernumerary mitral papillary muscles; fibrosis and shortening of mitral valve leaflets and chordae tendineae; hypertrophy of both cardiac ventricles; pronounced dilatation of right heart chambers; early atheroma of aorta and main pulmonary artery; generalized acute passive hyperemia; and recent contusion of soft tissues overlying the sternum.

Alteration of the Circulation: All available evidence points to an arteriovenous shunting via the two points of communication between the ventricles. The diversion of arterial blood from the arterial to the venous circuit could have been very considerable because of the large size of the openings. Clinical data are meager and we have no means of knowing whether or not there was a terminal reverse of blood flow. Shock due to trauma seems to have been the precipitating factor in the acute right heart failure, as is suggested by the great distention of the right side of the heart observed at autopsy.

Summary and Conclusions

Three new cases of cardiac malformation consisting of one or more communicating openings between the ventricles without any other anomalies of serious nature are added to the very small group already recorded in the literature.

All occurred in children between the ages of 6 and 12 years, two being males, the other a female.

Two display a single defect in the muscular division of the interventricular septum; the third shows in addition to this a defective pars membranacea, placing it in the clinical category of "maladie de Roger."

Cases 1 and 3 belong in the acyanotic group of congenital heart disease with transient or terminal reversal of blood flow. Case 2 is most remarkable, not because of any cardiac symptoms, for any appreciable shunting in either direction was impossible, but on account of its dissimilarity to the others herein described or in the literature. It is interesting, and from the standpoint of correct classification most important, that although the opening is a minute one it nevertheless possesses a complete lining of endocardium and for this reason may properly be termed a true congenital interventricular septal defect.

Evidence of arteriovenous shunting during life is plainly written in the hypertrophied right ventricular walls of Cases 1 and 3. While lamentably little is known about the lives of these children it would appear that the strain on the hearts in Cases 1 and 3 initiated by the septal imperfections was quite grave, for the 12 year old boy died suddenly while sitting in church and the 7 year

old girl succumbed to an injury in itself hardly sufficient to cause death. In the boy it is possible that emotional activity may have precipitated the acute heart failure; in the girl it is quite conceivable that trauma to the chest induced shock which could not be borne by the unbalanced heart. The heart murmur heard in Case 3, described by the examiner as a double sound over the mitral area, is of doubtful significance for typically the murmur produced by interventricular septal fenestra is holosystolic and very harsh.

All subjects escaped the most serious and frequently occurring complication of congenital heart disease (endocarditis) with the possible exception of the girl in Case 3, in whom the fibrosis of the mitral valve suggests healed rheumatic disease. Against this is the absence of histological changes attributable to rheumatic fever in the fibrotic valve or the myocardium. A more plausible explanation for the thickening is long continued trauma and strain on the valve due to its proximity to the defect in the pars membranacea where with each systole there could have been a splitting of the column of blood, thus simultaneously exposing both sides of the valve to wear and tear.

REFERENCES

1. McGinn, Sylvester. The incidence and progress in the recognition of congenital heart disease at the Massachusetts General Hospital. *J. Tech. Methods*, 1936, **16**, 98–102.

2. Abbott, Maude E. Atlas of Congenital Cardiac Disease. The American Heart Association, New York, 1936, 36.

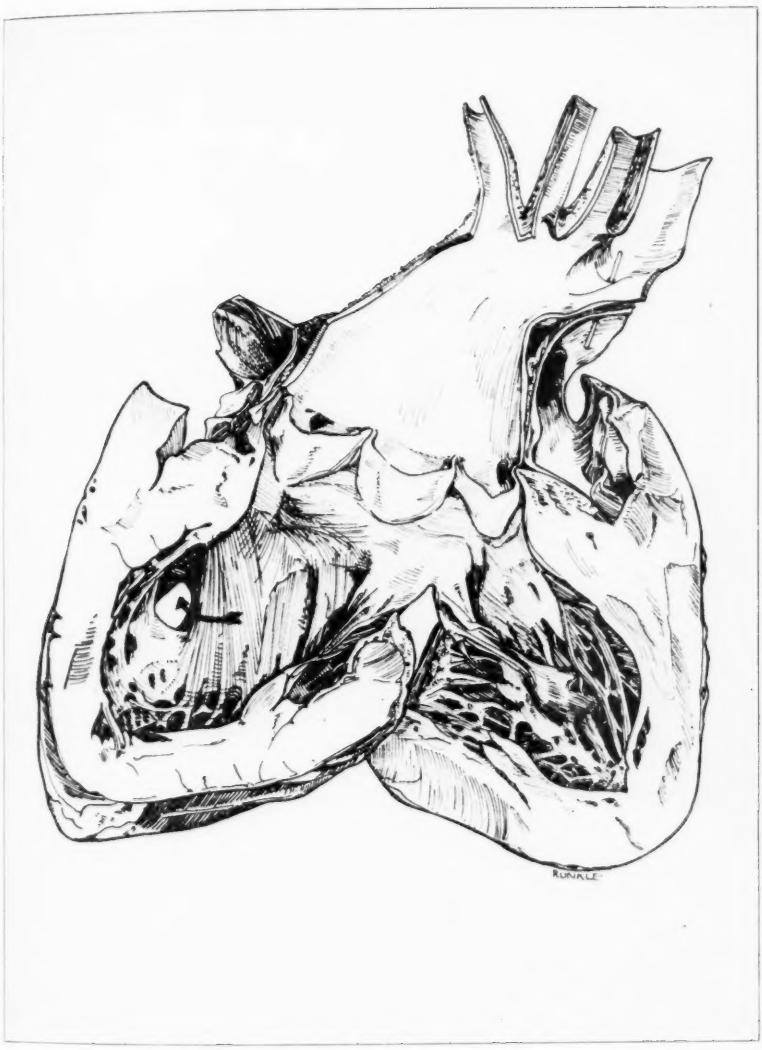
3. Abbott, Maude E., and Shanly, Eleanor. Cardiac defects in the light of the comparative anatomy of the vertebrate heart. *Internat. A. M. Museums Bull.*, 1922, **8**, 188–213.

4. Weiss, Edward. Congenital ventricular septal defect in a man, aged seventy-nine. *Arch. Int. Med.*, 1927, **39**, 705–709.

DESCRIPTION OF PLATES

PLATE 114

FIG. 1. Drawing of left side of heart from Case 1, showing position of interventricular septal defect, two of the three right coronary ostia, and the patent ductus arteriosus through which a bristle has been passed.



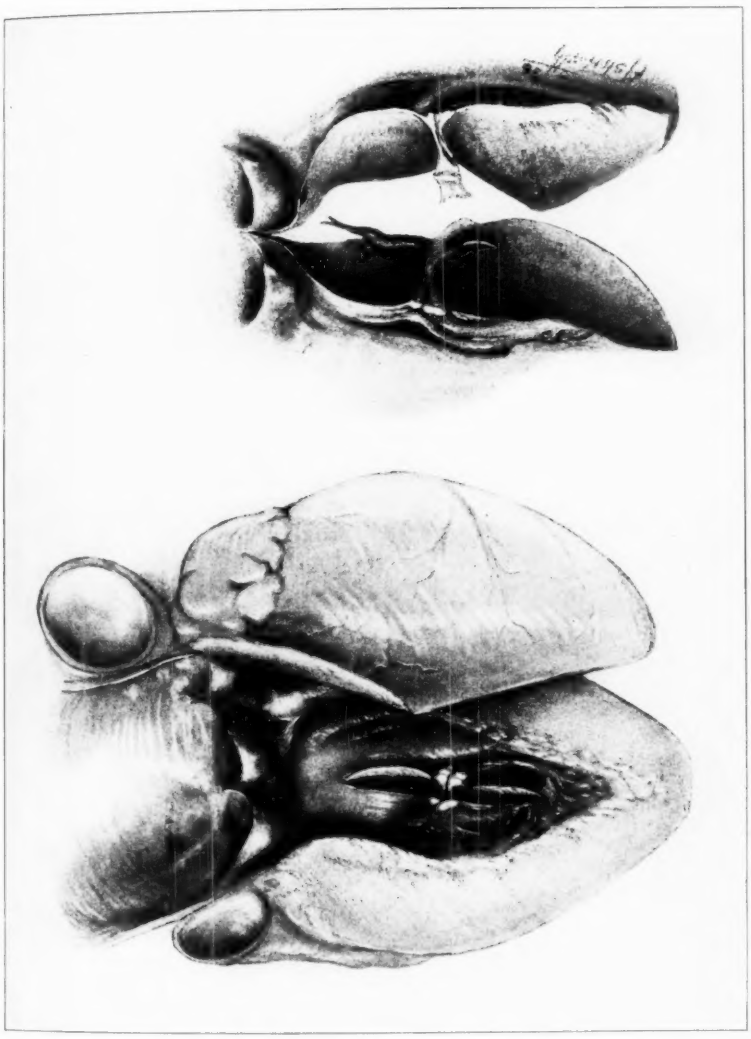
1

Mason and Hunter Defects of Cardiac Interventricular Septum

UNIV. OF MICH.

PLATE 115

FIG. 2. Two views of the endocardial tube from Case 2 filling the imperfection in the muscular segment of the interventricular septum. A portion of the channel has been exposed by means of a shallow incision in the left aspect of the septum. The entire course of the opening is shown in the smaller drawing in which the sagittal cut has been completed and the margins everted. Note the pearly character of the endocardium about the left terminus of the defect.



Mason and Hunter Defects of Cardiac Interventricular Septum

UNIV. OF MICH.

PLATE 116

FIG. 3. In this drawing of the heart reported in Case 3 the right chambers have been opened and widely everted in order to bring the two communications into full view. Interrupting the tricuspid valve is the larger opening at the position of the pars membranacea; in the background beyond this is the margin of the anterior mitral cusp and one of its chordae tendineae, accounting for the small fenestration. The ovoid defect in the muscular division of the septum is separated from that in the membranous septum by a considerable amount of muscle.

3

Mason and Hunter Defects of Cardiac Interventricular Septum

UNIV. OF MICH.

HYPERPLASIA AND REGENERATION OF THE MYOCARDIUM IN INFANTS AND IN CHILDREN *

H. EDWARD MACMAHON, M.D.

(From the Department of Pathology, Tufts College Medical School, Boston, Mass.)

The purpose of this paper is twofold: first, to point out that in cardiac hypertrophy of infants there may be an active proliferation of myocardial elements in addition to growth by enlargement of the individual muscle fibers; and secondly, to prove that during childhood heart muscle fibers may regenerate following severe injury. Both of these observations are in contradiction to current opinion. In textbooks and journals it is stated that the increase in size of the heart in cases of cardiac hypertrophy is due to an increase in size of the individual muscle fibers, and is not the result of a multiplication of these. Furthermore, it is almost axiomatic that the one and only form of myocardial healing subsequent to necrosis of heart muscle fibers is by the proliferation of connective tissue with ultimate repair by fibrosis. The former hypothesis in all likelihood holds true for the enlarged hearts of adults irrespective of cause, and is based primarily on the following two reasonably well established facts. First, careful measurements of the muscle fibers of hypertrophied hearts of adults have shown that an increase in size and weight of the heart may be explained mathematically on the basis of an increase in size of the individual myocardial fibers. Secondly, patient search throughout the myocardium in such cases of cardiac hypertrophy has failed to reveal any positive evidence, in the form of mitoses, of true myocardial proliferation. This frequently recorded absence of mitoses is also the most important single fact on which the statement that heart muscle fibers can not regenerate is based.

MYOCARDIAL HYPERPLASIA

It is rather difficult to determine at what age an infant's heart may be considered to have acquired its full complement of muscle fibers. It is generally believed that at the time of birth of a full-

* Received for publication May 26, 1937.

term child, the heart, though less than a tenth of its adult size, is completely formed. The rarity with which a single mitotic figure is found in average sized hearts, even at birth and during the first month of life, lends weight to this viewpoint and strongly suggests that an active proliferation of muscle elements under ordinary conditions ceases quite early. The increase in size of the infant's heart from this period to adult life, estimated by a progressive gain in weight, may be readily accounted for by an increase in size of the individual muscle fibers. The chance finding of an isolated mitosis in a heart muscle fiber of an average sized heart of a newborn infant is probably nothing more than a sign of a normal but somewhat delayed physiological process. In contrast to this the presence of many mitoses in heart muscle fibers of enlarged hearts in infants of 1 and 2 years of age, though probably nothing more than an acceleration of this normal growing process, may be justifiably regarded as a type of hyperplasia. This possibility of an increased proliferation of heart muscle fibers has been entirely overlooked as a factor in the histiogenesis of cardiac hypertrophy of infants.

A study of this problem of the normal and pathological growth of heart muscle elements was prompted by the finding of an unusually large number of mitoses in the myocardial fibers of an enlarged heart of a 6 months old child. This child died in uremia, the result of an anomalous development of the mucosa in both ureters, in which valve-like folds were formed causing complete intermittent urinary obstruction. The heart of this child was well formed. It weighed 60 gm., which is just twice the average weight for a child of this age and size. It showed particularly an enlarged left ventricle with an increase in thickness of the left ventricular wall. In a careful histological examination of two other enlarged hearts from infants of 12 and 20 months of age respectively — comprising a case of so-called primary idiopathic hypertrophy, as well as a case of cardiac hypertrophy secondary to coarctation of the aorta — it has also been possible to demonstrate the presence in heart muscle fibers of mitoses in all stages of nuclear division. The debatable interpretation of this finding as to whether it should be considered as true hyperplasia or merely a somewhat more active stage in the natural histiogenesis of the myocardium would seem to be of secondary importance. More important is

the indisputable fact that during this early age growth of the myocardium by an active proliferation of muscle elements may and does take place in cardiac hypertrophy of infancy.

The histological appearance of the myocardium in cases of cardiac hypertrophy in infancy varies. In the case of congenital idiopathic hypertrophy the increase in size of the individual fibers was scarcely perceptible. The fibers showed normal branching and well developed myofibrils. Mitoses were seen in the muscle fibers in the peripheral portion of the myocardium. In the 2 cases of secondary hypertrophy associated with coarctation of the aorta and diminished urinary elimination, the muscle fibers were distinctly larger than normal and the myofibrils were increased in number. This true hypertrophy was not found absolutely uniformly throughout the ventricular wall, for at times small delicate fibers and large thick fibers were seen lying side by side. In these 2 cases, in addition to an increase in size of the muscle fibers, mitoses were also found. They were most numerous toward the periphery and toward the tip of the left ventricle. Both cases showed normal myocardial branching. The fibers were regularly arranged and transverse striations throughout the myofibrils were everywhere seen. At this age, no transverse lines of segmentation, intercalated discs, have yet appeared.

The division of the nucleus occurs at the center of the fiber (Fig. 1). All stages of mitotic division are recognizable, including the single spireme, the monaster, diaster and double spireme (Figs. 2, 3 and 4). At the site of nuclear division one centrosome is seen at the end of each spindle (Fig. 5). The spindle is large and long search may be necessary to find one entirely intact (Fig. 6); serial sections are helpful in distinguishing questionable mitoses. The chromatin material appears as clumps of granules and short rods and stains intensely with hematoxylin. This chromatin material in a dividing nucleus may be scattered rather irregularly over the spindle (Figs. 2 and 3). The cytoplasm at the site of nuclear division is comparatively clear and is free of myofibrils (Fig. 6). Usually myofibrils showing transverse striations are seen at each side of the spindle, while just beyond each end of the dividing nucleus the cytoplasm becomes more intensely stained and delicate transverse striations, best demonstrated by Mallory's phosphotungstic acid hematoxylin stain, are visible in the myoplasm be-

fore longitudinal fibrils in this area are clearly recognizable. This differentiation of these transverse striations in the myoplasm appears to represent the early growth and development of the myofibril in the proximity of the dividing nucleus.

Myocardial Regeneration

During the preparation of this paper, which originally was planned to include only the problem of myocardial proliferation in cardiac hypertrophy in infancy, my attention was called by Dr. F. B. Mallory to an autopsy that was performed under his supervision in 1916. A boy, 6 years of age, had died several days after the onset of diphtheria. The most striking finding at the postmortem was the presence of a dilated heart showing a yellowish discoloration of the myocardium. A careful histological examination of the heart muscle showed severe degenerative changes with necrosis of the muscle fibers. This was associated with edema of the stroma and a moderate exudate of endothelial leukocytes and lymphocytes. There was an early proliferation of connective tissue of the myocardium. Most interesting, however, was the presence of isolated mitotic figures in heart muscle fibers bordering these zones of destruction (Fig. 7). This case appears to be the first to have been recognized as showing positive evidence, in the form of mitoses, of true myocardial regeneration. This is apparently a most unusual finding and yet its presence is absolute proof that regeneration may occur. Such observations as this point to the necessity of a more careful histological study of this condition in children. Moreover, it probably explains, in part at least, the frequent absence of myocardial scarring in hearts of children who, months or years earlier, had suffered severe myocardial injury. Finally, it supports from a histological standpoint the recognized need of prolonged rest during the period of recovery and repair following severe myocardial injury; and at the same time it offers the possibility and hope of a *restitutio ad integrum* subsequent to necrosis of myocardial fibers in children.

Review of Literature

To review the rather extensive literature dealing with the subject of the growth and regeneration of heart muscle fibers it is necessary to begin with a number of papers that appeared about

the middle of the last century. At that time the development and use of the microscope was limited and the methods used in the preparation of sections for histological study were comparatively poor. For these reasons it is difficult to evaluate the conclusions drawn in these early papers, for often these were based much more on theory than fact. Goldenberg in 1886 reviewed the literature on this subject since 1850, discussing the earlier papers of Kölliker,[1] Foerster,[2] Vogel,[3] von Rokitansky,[4] Friedreich,[5] Paget,[6] and Rindfleisch,[7] a group of investigators who considered the hyperplasia of muscle fibers in cardiac hypertrophy merely as an accepted fact. It is of interest that Lebert [8] as early as 1857 pointed out that there was no histological basis for this point of view. Goldenberg [9] believed primarily in the hypertrophy of the individual muscle fibers as the best explanation for myocardial hypertrophy. He said, however, that hyperplasia by longitudinal splitting could occur. Considerable importance was attached to a paper appearing in 1875 by Zielonko,[10] a student of Virchow. This investigator, working on the hearts of frogs and rabbits, came to the conclusion that the hypertrophy of the heart was not merely the result of an enlargement of individual muscle fibers, but probably the outcome of cellular hyperplasia as well. This hypothesis was transferred at once from animal to man to explain the myocardial hypertrophy of the human heart, but it lacked proof. No proliferation had been seen and the idea was based on a second and equally questionable hypothesis, "that hypertrophies are greatest in the young and middle ages, because during this period physiological development is still going on, and growing stimuli are at hand." Tangl,[11] after making careful measurements on heart muscle fibers, and after failing to find a single mitosis in a study of hearts showing cardiac hypertrophy, came to the conclusion that hypertrophy of the heart was to be explained entirely by a hypertrophy of the muscle elements. Ziegler [12] adopted a similar view but added that it is possible that the number of muscle cells are increased as well. Wideröe [13] also could find no mitoses and believed that in the absence of mitoses one could reasonably conclude that in cases of cardiac hypertrophy one was dealing simply with a hypertrophy of muscle fibers. Adami and Nicholls,[14] in speaking of hypertrophy of the myocardium, say, "Microscopically the muscle fibres are increased in

thickness and probably in number," but found no absolute evidence of true proliferation.

The idea of myocardial proliferation gained some support from a group of investigators who studied the repair process following myocarditis. They believed they saw signs of active regeneration following severe injury. Saltykow [15] in 1908 made the statement that in both acute and chronic myocarditis the muscle elements play a very important rôle in regeneration, with the result that there is a formation of new myocardial fibers. No mitoses were found in any of these cases. This paper was supported by Heller,[16] who made many drawings of muscle fibers, showing variations in size and shape of the nuclei, but again no mention was made of the presence of mitoses. Warthin,[17] as recently as 1924, believed he saw signs of muscle regeneration in 3 of 15 cases of diphtheritic myocarditis and said, "Near the necrotic or degenerated portion of the heart muscle, the nuclei show great variety in size and form. They increase in length and show evidence of longitudinal splitting in every possible stage of division. The living muscle substance bordering the injured area also undergoes longitudinal splitting into muscle bands containing nuclei. These bands grow into the perimysial tubes filling these up, replacing cell detritus and connecting with the living muscle on the other side of the defect. Muscle bands without nuclei, but accompanied by myoplastic nuclei, also extend into the tubes occupied by the dead muscle substance. These bands lie at the periphery of the tube, and in some cases appear to form a hollow cylinder enclosing the remains of dead muscle substance." No mention is made here by Warthin of even a single mitotic figure.

The more recent papers of Collier [18] and Karsner, Saphir and Todd [19] conclusively pointed out that in myocardial hypertrophy there is an increase in width and length of the sarcomere, or in other words, a hypertrophy of the muscle fiber. These latter investigators repeating the work of Goldenberg and Tangl made careful measurements of the muscle fibers of both hypertrophied and atrophied hearts of adults. They considered that the number of nuclei per field in an atrophic heart is increased, but a most careful search failed to disclose a single mitotic figure. They added, however, "It is possible that since the hearts were obtained postmortem, any mitotic figures present might have been com-

pleted." Kaufmann,[20] Aschoff,[21] and Mönckeberg [22] also stated emphatically that in myocardial hypertrophy the fibers increase in volume with an increase in the number of myofibrils and a proportionate increase in the size of the nucleus. There is no proliferation with an increase in the number of fibers, and mitotic division of the nuclei is absent. MacCallum,[23] and Boyd [24] in their texts likewise subscribe to this view. Mention of the possible proliferation of myocardial fibers in infancy was made by Rössle [25] who said, "With age the fibers increase in thickness, the number of bundles increases, fibers increase in length and muscle elements increase after birth." The possibility of regeneration of muscle elements was supported by Mallory [26] who stated that in very young children there is a general hyperplasia of muscle elements following injury.

Summary

Evidence is presented in the form of mitotic division of the nuclei of heart muscle fibers to indicate first, that in cardiac hypertrophy of infants a proliferation of heart muscle fibers can take place, and secondly, that in severe myocardial injury in children, regeneration of myocardial elements can occur.

REFERENCES

1. Kölliker, A. Handbuch der Gewebelehre des Menschen, für Aerzte und Studirende. Wilhelm Engelmann, Leipzig, 1852.
2. Foerster, A. Handbuch der speciellen pathologischen Anatomie. Leopold Voss, Leipzig, 1852.
3. Vogel, Julius. Pathologische Anatomie des menschlichen Körpers. Vom Baue des menschlichen Körpers, von Sömmerring, Samuel Thomas. Leopold Voss, Leipzig, 1845, **8**, Pt. 1, 154.
4. Von Rokitansky, Carl. Lehrbuch der pathologischen Anatomie. Braumüller, Vienna, 1856–61, Ed. 3, **2**, 215.
5. Friedreich, Nikolaus. Krankheiten des Herzens. Handbuch der speciellen Pathologie und Therapie, Virchow, R. Erlangen, 1855, **2**, 153–429.
6. Paget, James. Lectures on Surgical Pathology. Lindsay & Blakiston, Philadelphia, 1865, Ed. 3.
7. Rindfleisch, Georg Eduard. Lehrbuch der pathologischen Gewebelehre. W. Engelmann, Leipzig, 1867–69.

8. Lebert, H. Traité d'anatomie pathologique. J.-B. Baillière, Paris, 1857-1861.

9. Goldenberg, B. Ueber Atrophie und Hypertrophie der Muskelfasern des Herzens. *Virchows Arch. f. path. Anat.*, 1886, **103**, 88–130.

10. Zielonko, J. Pathologisch-anatomische und experimentelle Studien über Hypertrophie des Herzens. *Virchows Arch. f. path. Anat.*, 1875, **62**, 29–57.

11. Tangl, Franz. Ueber die Hypertrophie und das physiologische Wachstum des Herzens. *Virchows Arch. f. path. Anat.*, 1889, **116**, 432–456.

12. Ziegler, Ernst. Lehrbuch der allgemeinen und speciellen pathologischen Anatomie und Pathogenese. Gustav Fischer, Jena, 1889, Ed. 6, **2**, 34.

13. Wideröe, Sofus. Histologische Studien über die Muskulatur des Herzens. *Virchows Arch. f. path. Anat.*, 1911, **204**, 190–196.

14. Adami, J. George, and Nicholls, Albert G. The Principles of Pathology. Lea & Febiger, Philadelphia, 1909, **2**, 158.

15. Saltykow, S. Über diffuse Myokarditis. *Virchows Arch. f. path. Anat.*, 1905, **182**, 1–39.

16. Heller, Arnold. Über die Regeneration des Herzmuskels. *Beitr. z. path. Anat. u. z. allg. Pathol.*, 1913–14, **57**, 223–231.

17. Warthin, Aldred Scott. The myocardial lesions of diphtheria. *J. Infect. Dis.*, 1924, **35**, 32–66.

18. Collier, William Dean. Adaptive changes of heart muscle. *J. M. Research*, 1922, **43**, 207–251.

19. Karsner, Howard T., Saphir, Otto, and Todd, T. Wingate. The state of the cardiac muscle in hypertrophy and atrophy. *Am. J. Path.*, 1925, **1**, 351–371.

20. Kaufmann, Eduard. Lehrbuch der speziellen pathologischen Anatomie für Studierende und Ärzte. Walter de Gruyter & Co., Berlin and Leipzig, 1928, Ed. 7 and 8, **1**, 56.

21. Aschoff, L. Pathologische Anatomie. Gustav Fischer, Jena, 1936, Ed. 8, **2**, 37.

22. Mönckeberg, J. G. Die Erkrankungen des Myokards und des spezifischen Muskelsystems. Handbuch der speziellen pathologischen Anatomie und Histologie, Henke, F., and Lubarsch, O. J. Springer, Berlin, 1924, **2**, 368.

23. MacCallum, W. G. A Textbook of Pathology. W. B. Saunders Company, Philadelphia, 1936, Ed. 6, 470.

24. Boyd, William. A Text-Book of Pathology. Lea & Febiger, Philadelphia, 1934, Ed. 2, 403.

25. Rössle, Robert. Wachstum und Altern. Zur Physiologie und Pathologie der postfötalen Entwickelung. J. F. Bergmann, München, 1923, 83.

26. Mallory, Frank B. The Principles of Pathologic Histology. W. B. Saunders Company, Philadelphia, 1914, Ed. 1, 432.

DESCRIPTION OF PLATES

PLATE 117

FIG. 1. Section of myocardium showing three mitotic figures in heart muscle fibers. The fibers vary in width and show branching. Capillaries are dilated. There is an increase in fluid in the intercellular spaces. Myocardial hyperplasia, congestion and edema. × 330.

FIG. 2. One of the three mitotic figures from Fig. 1 at greater magnification. This nucleus is cut slightly on the bias; the small circle is just below one pole of the spindle, and the chromatin material appearing as rods and granules is clustered about the spindle. No definite arrangement of the chromatin can be distinguished. The cytoplasm immediately adjacent to the spindle is comparatively clear, rather finely granular and free of both transverse and longitudinal striation. Myofibrils showing transverse striation are visible at both sides of the dividing nucleus. × 740.

FIG. 3. One of the three mitoses seen in Fig. 1 at higher magnification. A spindle is not visible. The chromatin appears as rods and granules unevenly distributed, forming in the center a rather coarse clump. The cytoplasm at the site of nuclear division is comparatively clear. At each side myofibrils showing cross striations are visible. × 740.

1

2

3

MacMahon Hyperplasia and Regeneration of Myocardium

UNIV. OF MICH.

PLATE 118

FIG. 4. One of the three mitoses seen in Fig. 1, more highly magnified. The mitotic figure is incomplete. No spindle can be distinguished. The chromatin is forming an incomplete circle, appearing as short rods and granules. A comparatively clear zone completely surrounds the dividing nucleus. Myofibrils showing transverse striations are distinguishable laterally and above and below. × 740.

FIG. 5. Section of myocardium, showing a mitotic division of a nucleus of a heart muscle fiber. A complete spindle with centrosomes is visible. The chromatin is arranged at the center, forming two rather even rows of coarse granules and rods (monaster). The cytoplasm at the site of nuclear division is clear and very finely granular. Laterally a delicate, nonstriated membrane bounds the muscle fiber. Above and below the nucleus the cytoplasm is denser, stains more deeply, and transverse striations appear before individual myofibrils are recognizable. × 740.

FIG. 6. Section of myocardium, showing a mitotic figure in nucleus of heart muscle fiber. The entire spindle is recognizable, including one centrosome. The chromatin appearing as clumps of granules and short rods is gathered about the center of the spindle to form an equatorial plate. There is a clear zone about the nucleus. Myofibrils showing transverse striations are seen at each side of the nucleus. Above and below the nucleus the cytoplasm is more intensely stained and transverse striations are visible in the myoplasm before longitudinal fibrils are clearly seen. × 740.

4

5

6

MacMahon Hyperplasia and Regeneration of Myocardium

UNIV. OF MICH.

PLATE 119

FIG. 7. Section of myocardium from a 6 year old boy who died from diphtheria. In the center of the field is a segment of muscle fiber showing a nucleus in an early stage of mitotic division. The chromatin appears as coarse clumps and short rods and is gathered to form an irregular spireme. At this stage no spindle is recognizable. Capillaries are dilated and the tissue shows both a fluid and cellular inflammatory exudate. (BCH–A–16–66, Dr. Mallory.) × 2000.

7

MacMahon Hyperplasia and Regeneration of Myocardium

UNIV. OF MICH.

MORPHOLOGICAL CHANGES IN THE SUPERIOR VENA CAVA AND RIGHT AURICLE IN RHEUMATIC HEART DISEASE *

ERIK WAALER, M.D.†

(From the Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, N. Y.)

According to the records of this laboratory, in a few cases of rheumatic heart disease changes were observed in the superior vena cava. These changes were localized at the junction of the vein and the right auricle, and were characterized by yellowish plaques in the intima and media with little or no inflammatory reaction. It was thought desirable to study systematically a larger group of cases, in order to ascertain the nature of the morphological changes, the frequency of their incidence, and whether they occurred only in the presence of rheumatic heart disease.

Seventy-three cases showing definite rheumatic endocarditis were selected for the study. Hearts with superimposed bacterial endocarditis were not included, in order to avoid the possibility of confusion with inflammatory changes of non-rheumatic origin. The ages of the patients ranged from 3 to 66 years. The majority of the patients died from the effects of rheumatic heart disease. In a few cases, individuals dying from other causes, the rheumatic carditis was clinically unsuspected. A control series of 15 hearts without any evidence of rheumatic disease was studied for comparison. In 10 of these the heart was normal, while in 5 there was hypertrophy secondary to chronic nephritis or arteriosclerosis. The ages of the patients in the control group ranged from 4 to 65 years.

Blocks were taken from the superior vena cava with the adjacent portion of the right auricle, and were fixed in Zenker's fluid without the addition of acetic acid or formalin. The sections were stained with hematoxylin and eosin, and by Weigert's elastic tissue stain combined with van Gieson's picro-fuchsin.

The lesions found in the cardiovascular system are shown in

* Received for publication May 27, 1937.

† Travelling Fellow, Malthe and Pasteur Foundations, Oslo, Norway.

Table I. In 16 of the hearts there were definite lesions in the superior vena cava. Among these 16 the changes were interpreted as rheumatic in 8, whereas the nature of the lesions in the other 8 was difficult to evaluate. In both groups the lesions were found in the endocardium at the junction of the superior vena cava, sometimes more towards the auricular side, at other times more to the venous side. In 57 of the cases there were no demonstrable changes in this region.

The macroscopic changes in the first group of 8 cases interpreted as being rheumatic in nature were as follows: In 4 cases the endocardium was slightly wrinkled and thickened over areas varying from 2 to 5 mm. in diameter. These plaques were yellowish in color and the surface was dull and slightly irregular. In 2 cases tiny, dull grayish yellow, slightly elevated verrucae 1 to 2 mm. in diameter were present. In 2 cases no gross lesions were evident and the lesions were found only microscopically (Table II).

The histological alterations were as follows: The intima, media and adventitia were thickened, partly because of increase in connective tissue, and partly because of swelling of the collagenous fibers. In addition there was slight infiltration of cells. Many of these cells were large with a faintly blue cytoplasm in hematoxylin-eosin preparations; their nuclei were vesicular and oval, and some contained a large stellate nucleolus. These cells had the usual characteristics of the Aschoff cells. In the outer third of the media a few of these cells were diffusely scattered. In the adventitia they formed typical Aschoff nodules which in some instances extended into the media (Fig. 1). In addition to these larger cells there were a moderate number of lymphocytes, plasma cells, a few polymorphonuclear leukocytes and cells with distorted elongate nuclei (Figs. 2 and 5). The long axis of these nuclei was usually directed perpendicular to the surface. This cellular infiltration was particularly increased in the outer third of the media, but in some instances the superficial layers of the intima were involved. Mainly lymphocytes and plasma cells were found in this region. In some cases the cellular infiltration and thickening of the intima and media were inconspicuous and the main finding was the Aschoff nodules in the adventitia (Fig. 3). As a rule there was an increased diffuse infiltration of lymphocytes in the adventitia.

In elastic tissue preparations the elastic fibers in the intima were seen to be spread apart and fragmented at the site of the cellular infiltrations. The internal elastic lamella was often interrupted and distorted, or was split into a fine meshwork of elastic fibers. The elastic fibers were always arranged parallel to the surface. In some areas loss of smooth muscle elements and increase of connective tissue were noted in the media.

The inflammatory changes described above have thus some of the features of the auricular rheumatic endocarditis described by MacCallum,[1, 2] and by VonGlahn,[3] and have also certain characteristics in common with rheumatic aortitis (Pappenheimer and VonGlahn [4]). However, the lesions are not so extensive in this region as in the left auricle and the aorta.

In the other group of 8 hearts the alterations were different. The intima was definitely thickened with one or more distinct yellowish plaques measuring 4 to 5 mm. in greatest diameter. The surface of the plaques was smooth and glistening. In 1 heart there was no distinct plaque but the intima was more diffusely thickened over an area measuring 2 by 1.5 cm. (Table II). The thickening of the intima was due to collagenous tissue (Fig. 4). No inflammatory reaction was noted within the plaques, but in the deeper portion of the media and the adventitia, in some instances, lymphocytic infiltrations were found; in others no cellular accumulations were present. There were many fine elastic fibrils in the thickened intima, which lay parallel to the surface. In most instances the media was thinned out beneath the plaque and showed a definite loss of elastic fibers and smooth muscle.

It is difficult to evaluate the exact nature of these plaques. In one instance Aschoff nodules were found in the adventitia beneath the plaques, but these do not justify any conclusion as to the nature of the changes found in the plaque. In the cases with lymphocytic infiltrations of the adventitia and deeper portion of the media the reaction was not necessarily of rheumatic origin. It is possible that the plaques represent the end stage of the acute rheumatic lesions described above. This, however, cannot be proved as no intermediate stage between the plaques and the acute rheumatic lesions were found. The supposition that the plaques represent healed lesions is supported by some of the data given in Table II. The patients with the rheumatic lesions were younger

than those in the other group; 6 were under 20 years, whereas only 2 were under 20 years of age in the group with the plaques. Furthermore, 3 of the patients with the rheumatic lesions had a very short rheumatic history and all of them showed either clinically or anatomically an acute exacerbation of their rheumatic condition before death. In the other group with the plaques all except 1 had had longstanding rheumatic heart disease with increasing symptoms of cardiac insufficiency. On the other hand, all these 8 patients had had chronic passive congestion, and it is therefore possible that the plaques were only sclerotic in nature, belonging to the type of changes called phlebosclerosis. The plaques did not contain any fat, so they cannot be regarded as atherosclerotic. Our control group includes too few cases to permit the conclusion that these plaques are found only in rheumatic heart disease.

One feature of interest is revealed in Table II, namely that most of the patients with the rheumatic lesion in the superior vena cava were females. No satisfactory explanation can be given for this.

The acute rheumatic lesions found in 8 cases may be regarded as a special localization of an acute rheumatic process. Lesions of rheumatic nature in the superior vena cava have been observed by Klinge,[5] but they were localized to the more peripheral parts of the vessel. In his paper on rheumatic auriculitis VonGlahn mentions only 1 instance of rheumatic auriculitis in the right auricle. In that case a chain of tiny verrucae was present along the anterior margin of the fossa ovalis. Some of the cases studied in this series showed very small verrucae, both in the neighborhood of the fossa ovalis and in the region of the superior vena cava. Apparently the rheumatic lesions show some preference for these regions of the right auricle.

The incidence of this rheumatic lesion is, as shown in Table I, greater than the valvulitis of the pulmonic valve, rheumatic aortitis or arteritis.

In the group of 15 control hearts no lesions similar to those described above were found.

Summary and Conclusions

In a study of the superior vena cava at its entrance into the right auricle rheumatic lesions were found in 8 out of 73 cases of

TABLE I

Localization of Lesions in 73 Cases of Rheumatic Heart Disease

Rheumatic lesions found at routine autopsy										Superior vena cava	
Total No.	Mitral valve	Aortic valve	Tricuspid valve	Pulmonary valve	Left auricle	Myocardium	Pericardium	Aortitis	Arteritis	Rheumatic	Unspecified
73	69	46	34	7	29	51	26	4	6	8	8

TABLE II

Sex and Age Distribution, Duration of Illness and Type of Gross Lesions in Superior Vena Cava Found in 16 Cases of Rheumatic Heart Disease

Autopsy No.	Sex	Age	History of rheumatism	Gross lesion
5089 Babies Hosp.	F	yrs. 9	6 mos.	Small wrinkled areas
6338 Babies Hosp.	F	11	6 mos.	Small verrucae
6389 Babies Hosp.	F	7	1 yr.	Small wrinkled areas
6456 Babies Hosp.	F	12	Attacks since age of 2 yrs.	Small wrinkled areas
11432 Presbyterian Hosp.	F	38	1 attack in childhood	Two verrucae
11524 Presbyterian Hosp.	F	17	Growing pains in childhood, now 6 mos. history	Small wrinkled area
11562 Presbyterian Hosp.	F	12	6 attacks since age of 5 yrs.	None
11580 Presbyterian Hosp.	M	35	Undiagnosed	None
6087 Babies Hosp.	M	8	3 attacks in 4 yrs.	Plaques
6187 Babies Hosp.	F	9	Second attack in 6 mos.	Plaques
11117 Presbyterian Hosp.	M	27	1 attack 11 yrs. ago	Plaques
11251 Presbyterian Hosp.	F	32	4 attacks in 8 yrs.	Plaques
11390 Presbyterian Hosp.	M	54	1 attack in childhood	Plaques
11536 Presbyterian Hosp.	F	36	1 attack 12 yrs. ago	Plaques
11543 Presbyterian Hosp.	F	35	Attacks 23 and 7 yrs. ago	Wrinkled yellowish thickening of intima
11697 Presbyterian Hosp.	F	36	6 attacks in 9 yrs.	Plaques

rheumatic carditis. The inflammatory reactions in the media and the adventitia were similar to those seen in the auriculitis and aortitis of rheumatic origin.

In 8 additional cases hyaline plaques were found. It could not be decided whether these plaques represented the final and healed stage of the acute rheumatic lesions, or whether they were sclerotic in nature and due to longstanding heart disease.

REFERENCES

1. MacCallum, W. G. Rheumatic lesions of the left auricle of the heart. *Bull. Johns Hopkins Hosp.*, 1924, **35**, 329.

2. MacCallum, W. G. Rheumatism. The Harrington lecture. *J. A. M. A.*, 1925, **84**, 21, 1545–1551.

3. VonGlahn, William C. Auricular endocarditis of rheumatic origin. *Am. J. Path.*, 1926, **2**, 1–14.

4. Pappenheimer, Alwin M., and VonGlahn, William C. Lesions of the aorta associated with acute rheumatic fever, and with chronic cardiac disease of rheumatic origin. *J. M. Research*, 1924, **44**, 489–505.

5. Klinge, Fritz. Der Rheumatismus. *Ergebn. d. allg. Pathol. u. path. Anat.*, 1933, **27**, 115–132.

DESCRIPTION OF PLATES

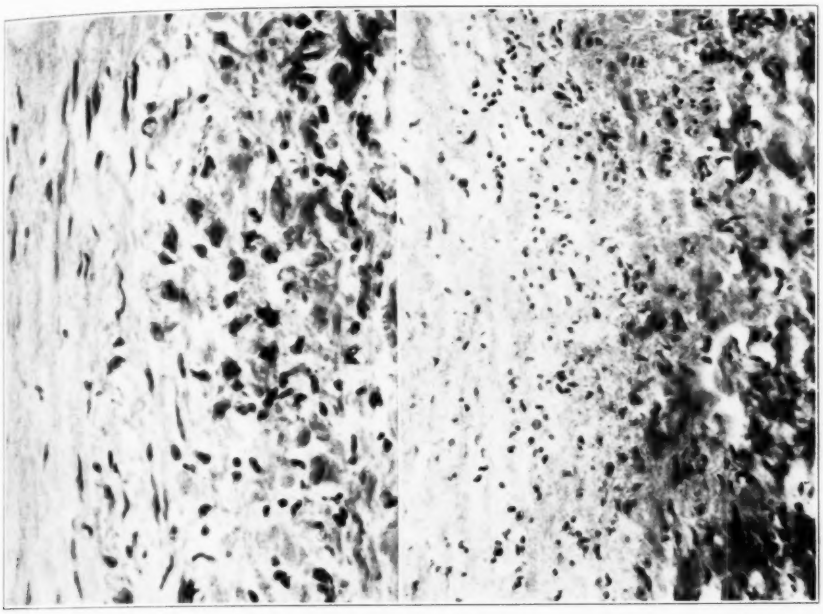
PLATE 120

FIG. 1. Autopsy No. 5989. Acute rheumatic endocarditis of right auricle at the entrance of the superior vena cava. Aschoff nodule in adventitia extending into the deeper portion of media. Hematoxylin-eosin stain.

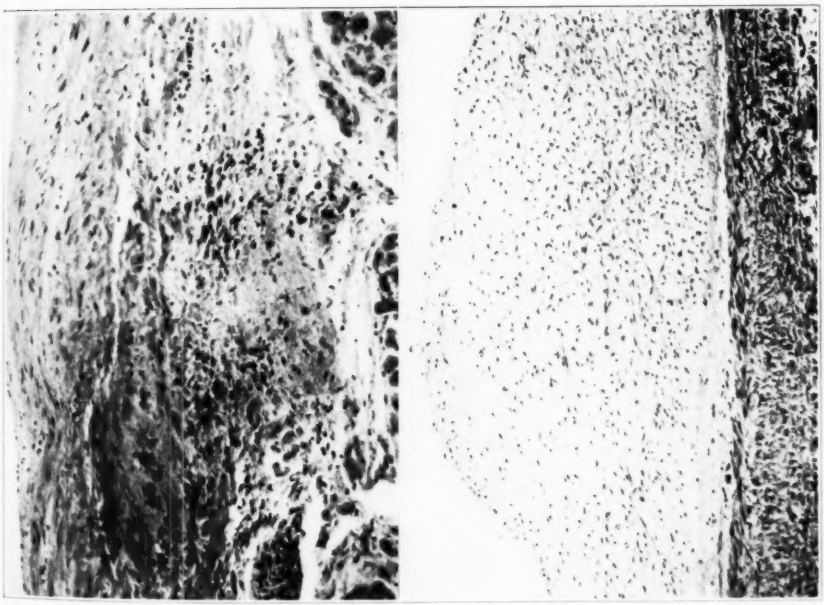
FIG. 2. Autopsy No. 5989. Acute rheumatic endocarditis of right auricle at the entrance of the superior vena cava. Thickening of media with cellular infiltration, lymphocytes, plasma cells, cells with elongate, distorted nuclei. Hematoxylin-eosin stain.

FIG. 3. Autopsy No. 11580. Acute rheumatic endocarditis of right auricle at the entrance of the superior vena cava. Aschoff nodules in adventitia. Hematoxylin-eosin stain.

FIG. 4. Autopsy No. 11117. Hyaline plaque in the superior vena cava at its entrance into the right auricle. Hematoxylin-eosin stain.



1 2



3 4

UNIV. OF MICH.

PLATE 121

FIG. 5. Autopsy No. 6338. Acute lesion of the superior vena cava at its entrance into right auricle. Thickening of media and adventitia and cellular infiltration. Hematoxylin-eosin stain.

5

Waaler Morphological Changes in Rheumatic Heart Disease

UNIV. OF MICH.

TORULA INFECTION *

J. T. Crone, M.D., A. F. DeGroat, M.D., and J. G. Wahlin, Ph.D.

(From the Departments of Neurology, Pathology and Bacteriology, University of Arkansas, School of Medicine, Little Rock, Ark.)

The existence of torulosis has been known to the medical profession for twenty years or more, yet the disease remains a distinct rarity. Some authors have expressed the opinion that this rarity is more apparent than real and that many cases are passing unrecognized. Nevertheless, there has been no great acceleration in the appearance of case reports which at present number about 50. Of these a few are of dubious authenticity. Although the cultural and morphological characteristics of the organism are fairly well established, animal inoculation experiments have been few, quite variable in result, and frequently incomplete. Because of these considerations certain phases of the literature are reviewed and another case is reported with observations on the bacteriology of the organism isolated and on the pathology of the disease produced in animals experimentally.

Stoddard and Cutler [1] separated torulosis from other yeast-like infections partly on histological grounds. The specific characteristics emphasized consisted of a solution of the tissues about the organism with little or no inflammatory reaction. The organisms were embedded in a gelatinous matrix. The reaction was chiefly monocytic. In the brain these changes resulted in the formation of pseudocysts. When the lesions were massive, caseation occurred. It is to be noted that in the latter event the lesions could hardly be regarded as specific. Subsequent experience in general confirms Stoddard and Cutler's description in all definitely proved cases. The brain lesions are remarkably uniform in character, but most authors describe granulomatous lesions in the meninges and other structures, giant cells being abundant. Necrosis with an exudation of polymorphonuclear leukocytes in the meninges has been reported,[2] and also caseation in the tracheobronchial lymph

* Received for publication June 7, 1937.

nodes,[3] but in cases without bacteriological control. In local exposed lesions a certain amount of suppuration seems to have been the rule.[4-6]

Torulosis was described originally and many times subsequently as a disease essentially of the central nervous system. However, the literature reveals four general classes of cases dependent on the distribution of the lesions: (1) encephalomeningitis alone; (2) central nervous system lesions as part of a more or less generalized torulosis; (3) visceral torulosis without localization in the brain and meninges; and (4) local infections.

Primary torulosis of the nervous system can be established as a reality only on the basis of a most meticulous postmortem examination. Hall, Hirsch and Mock [7] have furnished such a case. The presence of old lesions in the gasserian ganglion, absence of changes in the choroid plexus, and the absence of organisms or lesions in any other organs offering a portal of entry, as shown by cultures and histological examination, led the authors to the conclusion that the Torula had invaded the meninges directly from the nasopharynx. Five other less probant cases have been reported (Hansmann,[2] Freeman and Weidman,[8, 9] Swift and Bull,[10] and Barlow [11]).

More or less general infections with cerebral localization account for the greatest number of cases.[3, 9, 12-20]

The lungs have frequently been described as the principal or unique extracranial focus.[3, 9, 12-14, 20, 21] Other organs that have been involved are the liver (rarely), spleen, suprarenal glands, kidneys, testes, abdominal, thoracic and axillary lymph nodes, bone marrow and the periosteum.

Least known are visceral lesions without brain involvement. Examples are furnished by Gill,[4] Berghausen,[22] and Sheppe.[23] Particularly interesting is Gill's case, because with a less careful laboratory study it would have been classified as a purely local infection. There were abscesses in the ethmoid sinuses, the antrum and hard palate. The organisms were cultured from the blood. The patient recovered and is known to have remained well for 7 years. Sheppe's patient ran a protracted course with joint pains, sore throat and anemia, finally dying with a gelatinous torulous pneumonia. Berghausen's patient had a chronic ulcer of the tongue and died with an unproved pulmonary torulosis. If

torulosis is of more frequent occurrence than the literature reveals, it is in this group of cases and in the following group that failures in diagnosis were made.

There are few reports of local infection. The fact that Gill discovered 3 of these indicates that inadequate bacteriological studies may, in part at least, account for the apparent rarity of this type of case. In the 3 cases of Gill [4] and in the cases of Jones [5] the lesions were located in the nasopharynx or mouth, and had the characteristics of chronic abscesses and ulcers. McGehee and Michelson [6] observed chronic communicating pelvic and inguinal abscesses in a negress. All of these patients recovered.

A coincidence between Hodgkin's disease and torulosis was noted by Mallory (see Cabot's article [17]) who found the coincidence nearly as great as between Hodgkin's disease and tuberculosis. Fitchett and Weidman,[16] on the contrary, believe they have reported the only typical example of this association. The cases of Freeman and Weidman [8] and Smith and Crawford [24] are involved in some doubt.

In mycotic infections one looks for predisposing factors. Berghausen's [22] patient injured his tongue and in this wound the lesion developed. Previous ill health has been mentioned in the cases of Gill,[4] Freeman,[9] Fitchett and Weidman,[16] Cabot,[17] and Williams.[20] All cases seem to have occurred among members of the less fortunate strata of society.

The diagnosis of torulosis must rest on evidence other than clinical and physical findings. In some of these cases the diagnosis has been based on the histopathology alone.[2, 3, 18, 24-32] No cultures were reported by Johns and Attaway.[33] Isolation and identification of Torula histolytica is reported by the majority of workers.[5-8,11-17, 19-22, 34-41] Sheppe [23] presents an unusually complete study. The same is true of Urbach and Zack.[42] The cases of local torulosis reported by Alvarez [43] and Tabor [44] are not due to T. histolytica but to some species of pink Torula. Badham's case,[45] and that of Swift and Bull,[10] apparently are not cases of torulosis but rather some type of ascomycosis. One of Ball's cases [46] seems given with regard to acceptance of all Torula infections as being due to pathogenic species of genus Torula Hansen. Some European papers dealing with Torula infections, notably the French, to be a monilial infection. Finally a word of caution should be

concern themselves with an entirely different organism under the designation of genus Torula Persoon non Hansen.[47] Strictly speaking the latter is better taxonomy. It is unfortunate that these different usages should have occurred. The escape seems to be to drop the term Torula entirely and adopt the generic name suggested by Vuillemin,[48] *i.e.* Cryptococcus. In this paper we are concerned only with the counterpart of Hansen's Torula or the genus Cryptococcus of Vuillemin. To conform to current usage we will retain the name T. histolytica in this paper.

Animal inoculations that have been carried out since Stoddard and Cutler's study have contributed few new facts and have even obscured to some degree the specific character of the Torula lesion. Although originally declared unsuitable, guinea pigs have frequently been employed.[6, 8, 13, 15, 19, 35, 36] Massee and Rooney[15] obtained nodules in the peritoneum in a third of their animals. Lynch and Rose[35] and Watts[13] got negative results. Where the inoculations were positive the lesions were often hardly typical because of a well defined granulomatous reaction with many giant cells,[8, 19, 36] or again they were limited in extent.[8, 15] McGehee and Michelson[6] obtained frank abscesses. On the other hand, typical lesions have been produced in the brain.[8, 19] Rabbits have given irregular results, negative to intraperitoneal and positive to intravenous injections.[36] Sheppe[23] produced a gelatinous pneumonia by intratrachial injections. Fitchett and Weidman[16] inoculated a dog and Barlow[11] a cat intrathecally. Both animals succumbed. In the latter there was a marked inflammatory reaction and evidence that the Torulae were merely maintaining themselves. In neither animal did generalization occur. Fitchett and Weidman[16] and Weidman[34] inoculated monkeys subcutaneously and obtained abscesses at the site of the injection which healed rapidly. Fitchett and Weidman[16] obtained typical lesions in rats inoculated intrathecally. That is to say, there were widespread cyst-like lesions in various organs without inflammatory reaction other than a few endothelial leukocytes. Rappaport and Kaplan,[19] by intraperitoneal injection, produced typical brain cysts and also granulomatous lesions with many giant cells in other organs. In Jones'[5] experiment, rats that were inoculated intraperitoneally were living after 3 months. Torulae were recovered from nodules in the kidneys. Mice, which are the most susceptible laboratory

animals, have been employed only twice by the various investigators. In one instance results were negative, possibly because the material had been heated.[13] In the other, the lesions showed brain cysts and in all organs the typically feeble inflammatory reaction.[16] From a review of animal experimentation one is not impressed with the specificity of the lesions of torulosis except in the instances where cysts occurred in the brain.[8, 16, 19] Moreover, no satisfactory comparison of results can be made because of the variety of animals used, animals having presumably varying degrees of susceptibility. From this it follows that no information is available concerning the pathogenicity of different strains of Torulae.

Report of a Case

Clinical History: A male negro, aged 25 years, entered the hospital Nov. 27, 1935, complaining of weakness, violent headache and double vision. These symptoms had begun with headache sometime in October, 1935.

Examination revealed some mental clouding, moderate emaciation, great muscular weakness and fever. There was an external strabismus and diminution of vision. The optic discs, particularly the left, were red and somewhat swollen. The tendon and cutaneous reflexes were normal with the exception of the patellars which were absent. Physical findings were otherwise negative.

Laboratory Findings: Leukocytes 10,500 falling to 6250. Schilling count 0-1-0-1-7-70-10-11. The cerebrospinal fluid showed increased pressure, sugar negative, globulin +++, cell count 135. Numerous yeast-like cells, some of them budding, were present. The spinal fluid Wassermann was negative. The urine showed a trace of albumin, smear and culture positive for yeast-like organisms. (Details on blood and urine cultures appear elsewhere in this paper.)

During the subsequent 6 weeks delirium developed, followed by deepening coma with slow pulse and all the usual symptoms and signs of meningitis. Evidence of pulmonary consolidation appeared shortly before death, Dec. 15, 1935.

Postmortem Examination: The calvarium was removed in the usual manner. The dura was tense and red. When incised no cerebrospinal fluid escaped. There was only moderate flattening of the cerebral convolutions. The vessels were markedly engorged.

The leptomeninges of both brain and cord showed a diffuse thickening and cloudiness. This was most marked over the brain stem. Everywhere, but particularly along the cerebral vessels, were innumerable, minute yellow masses, none of them more than 1 mm. in diameter. In the sulci there was a small amount of yellowish exudate. After fixation of the brain the tubercle-like bodies were no longer visible, only the cloudiness of the meninges

remaining (Fig. 1). A series of frontal sections showed no appreciable dilatation of the ventricles. They were filled, however, with a colorless gelatinous material. About the lateral ventricles were thickly set cavities varying in diameter from 2 mm. down to the limits of visibility. These likewise contained a gelatinous material (Fig. 2). The choroid plexuses were thick, pale pink and somewhat translucent.

In the lower lobe of the right lung was a patchy gray consolidation and a fibrinous pleuritis. The extreme granularity of the cut surface suggested miliary tubercles with confluence in the denser areas.

Scattered yellow and pearly gray miliary nodules were found on the surface of the left suprarenal gland and throughout the parenchyma of both kidneys (Fig. 3). Lymph nodes were examined with particular care but no lesions were discovered here or in other organs.

Microscopic Findings: The leptomeninges were heavily infiltrated by large mononuclear cells and by a few lymphocytes. The majority of the former had the characteristics of compound granule cells and a few of them contained yeast-like organisms. Again there were collections of the organisms without cellular reaction. The lateral ventricles contained innumerable yeast cells and a few degenerating leukocytes, probably monocytes. The adjacent cyst-like structures, described grossly, were filled with yeast cells and a few large mononuclear cells having foamy cytoplasm. The borders of these spaces were ragged and showed no evidence whatever of proliferative or exudative changes. The smallest of them were simply expansions of the perivascular spaces and contained yeast cells alone. The meninges of the cord resembled those of the brain. In the white matter of the cord, about the posterior horns, were numerous mucin bodies. In both brain and cord the nerve cells were in varying degrees of degeneration, mostly mild.

The pulmonary consolidation proved to be a banal bronchopneumonia. The exudate was, however, unusually uniform and consisted almost uniquely of polymorphonuclear leukocytes and fibrin. Torulae, but no other organisms, were found after repeated search (Gram's stain). The Torulae were not believed to have caused the pneumonia.

The lesions in the suprarenal gland and in the kidneys consisted chiefly of punched out cavities containing one or more organisms. An inflammatory reaction was entirely absent or was represented by a few endothelial leukocytes (Fig. 4). Again, endothelial leukocytes and lymphocytes were sufficiently numerous to form a follicular lesion. Only one small Langhans' giant cell was found. Numerous renal tubules were dilated by masses of yeast cells (Fig. 5). In the pancreas there were minute areas of liquefaction necrosis containing yeast cells and strands of collagenous material (Fig. 6). Remarkable was the absence of degeneration immediately adjacent to the lesions. No changes related to the Torula occurred in the lymph nodes or spleen.

Laboratory Diagnosis

A centrifuged specimen of spinal fluid was examined in a hanging drop. A number of round bodies could be seen. Morphologically these were not unlike red blood cells except that an occasional one was budding. Cultures were made in 1 per cent dextrose veal infusion medium (Difco). After 48 hours incubation at 37° C. a surface growth appeared which on microscopic examination was seen to consist of budding yeast-like cells. Subcultured on Sabouraud's agar a growth was obtained in about 72 hours, which after about 14 days developed a light orange color. Two catheterized urine specimens were examined during the course of the disease. The sediment from the centrifuged specimens was seeded directly on Sabouraud's agar. In both instances growth identical with that obtained from the spinal fluid made its appearance. On two occasions similar organisms were recovered from the blood stream. Ten cc. of blood were inoculated into 50 cc. of brain infusion dextrose broth medium containing some calcium carbonate and brain particles in suspension. In 1 case the growth appeared after 10 days incubation and in another sample the blood culture was positive on the 15th day.

Morphology

Young cells varied in size from about 6 to 12 μ. In a hanging drop the cytoplasm appeared as a thin gray substance containing small granules. With age there appeared in the culture: (1) an

increase in the number of coarse granules in the cytoplasm; (2) formation of a hyaline cell content by the fusion of the granules; and (3) capsules or shells of varying sizes. These changes among others have recently been elaborated into a life cycle by Todd and Herrmann.[49] Based on the production of tube-like protuberances and fusion of cell contents, these authors believe that there exists a perfect stage in the life cycle of the Torula of yeast meningitis. In our preparations tube-like projections were frequently seen. At no time did we observe any fusion of cells. Empty shells were frequent. Cultures on gypsum blocks, sliced carrot, and Gorodkova's agar failed to yield ascospores. No mycelium was ever seen, even in cultures 1 year old. These facts have led us to conclude that the organism in question was Torula Hansen non Turpin, Persoon.

Cultural Characteristics

The Torulae that were isolated grew well on meat extract dextrose agar, Sabouraud's agar and less well on sugar-free agar. Growth was more rapid at 37° C. than at room temperature. Pigmentation could be seen after 7 to 14 days. Various tints appeared: yellow, orange, burnt orange, chocolate, and white colors were seen at some time or other. At no time was the color pink or red. One culture growing on Sabouraud's agar, after a period of 4 months, developed a chocolate brown color. About this time secondary colonies began to make their appearance on the surface of the brown growth. The secondaries were white at first but later turned light orange. Microscopically the cells from the two areas were not remarkably different. This behavior is no doubt an evidence of dissociation and indicates that pigment changes might also be a phase of this process. Up to date no slimy colonies have been encountered. Broth cultures never yielded rings or pellicles, but growth occurred at the bottom in the form of a more or less granular sediment.

Biochemical Behavior

After 4 days incubation at 37° C. acid but no gas appeared in the tubes containing dextrose and sucrose beef extract broth. In

maltose broth the organism developed at best only a very feeble acidity. No acid or gas was noted in broths containing levulose, lactose, galactose, arabinose, melezitose, xylose, raffinose, dextrin, mannitol, sorbitol, dulcitol, and salacin. Tests for acetyl-methyl-carbinol were negative and the organism failed to yield a positive methyl red test. No hydrogen sulphide was formed. This Torula failed to hemolyze human blood cells.

Thermal Death Point

The authors have found in the literature no record of any thermal death point determinations. Using a 48 hour dextrose agar culture of Torula suspended in 10 cc. of peptone broth, pH 7.2, it was found that the organisms were killed by heating in a water bath for 42 minutes at 50° C. At 60° C. the cells were killed in less than 5 minutes. The resistance of the Torula is therefore not unlike that of many of the non-sporeforming bacteria.

Animal Inoculation

As a preliminary experiment 3 mice were used. Each animal was given an intraperitoneal injection of 0.5 cc. of a 24 hour suspension of Torula grown on Sabouraud's agar. The turbidity of the injected suspension stood at No. 10 on the scale of the McFarland nephelometer. One of the animals died after 5 days, the 2nd was killed at this time, and the 3rd mouse lived 8 days. In none of these animals was there any gross pathology. Cultures from the peritoneum, brain, and heart blood yielded Torulae in the two instances where such cultures were made.

The experiment was repeated using 9 mice divided into three groups. Group I received intraperitoneally 0.5 cc. of a 24 hour Torula suspension equal in turbidity to No. 1 on the McFarland scale. This was the lightest suspension. Group II received 0.5 cc. of a similar suspension equal in turbidity to McFarland No. 5. This was an intermediate dose. Group III received an equal amount of a suspension corresponding to a turbidity of McFarland No. 10. This was the heaviest dose. The following table sets forth the survivals in this series of animals.

Group I	Survived *days*
Mouse No. 4	2
Mouse No. 5	17
Mouse No. 6	22
Group II	
Mouse No. 7	9 *
Mouse No. 8	2
Mouse No. 9	40
Group III	
Mouse No. 10	1
Mouse No. 11	2
Mouse No. 12	18

Two guinea pigs were each inoculated intraperitoneally with 1 cc. of a 24 hour growth of Torula equal in turbidity to McFarland No. 10. One pig died after 30 days and the other, while still apparently normal, was killed and both were autopsied.

Of the 12 mice inoculated, 10 were completely studied postmortem. Because neither the dose of organisms injected nor the survival period seemed to have any significant influence on the results, all are considered together.

Only 2 animals failed to show the typical lesions of torulosis. In 1 of these, which had survived 9 days, there were no noteworthy pathological changes. The other survived only 1 day and showed an "acute splenic tumor" due to intense congestion and to crowding of the sinusoids with polymorphonuclear leukocytes.

Positive inoculations resulted in lesions which were similar in all of the mice. Moreover these lesions were similar in most respects to those observed in human cases.

Gross changes were neither conspicuous nor characteristic. On the peritoneal surfaces there was a thin mucoid exudate and there was a gray mottling of the lungs.

Microscopically Torulae were found in nearly every organ and even in the skeletal muscle. The predilection of the Torulae for the nervous system was apparent because involvement of the brain and the meninges was constant. The brain lesions consisted

* Mouse No. 7 got its head caught in the cage and was partially strangled, but when released apparently recovered and lived 2 days before death intervened. It had been accidentally inoculated subcutaneously.

of cyst-like cavities of variable size which contained numerous Torulae. An inflammatory reaction was utterly absent (Fig. 7). The meninges contained numerous Torulae together with only a few mononuclear leukocytes. (The brain lesions in mouse No. 9 fell short of this description in being rare and extremely minute and containing some epithelioid cells.)

Microincineration of the alcohol-fixed brain revealed nothing in the cyst-like cavities except Torulae which appeared as bright rings in the dark field.

In the lungs Torulae were usually extremely numerous, filling most of the alveoli and occurring in the stroma. Present also were variable numbers of macrophages with abundant foamy cytoplasm and small dense nuclei (Fig. 8). Hyperemia was always moderate and edema was slight or absent.

The lesions in the liver were definitely granulomatous. There were minute collections of monocytes and epithelioid cells among which were rare Torulae. In the kidney, suprarenal gland, spleen, myocardium and skeletal muscle the Torulae lay singly or in small groups in clear spaces in the interstitial tissues with no trace of an inflammatory reaction about them. The omentum and mesenteries contained enormous numbers of organisms with few or many macrophages. The macrophages were foamy, similar to those observed in the lungs. Of the above tissues and organs, the peritoneum, brain, and lungs were involved in all the animals.

In the guinea pig that was sacrificed no lesions were found. The one that succumbed showed lesions similar to those described in mice. There were large brain cysts but in other organs the lesions were much less extensive.

Concerning the appearances of the Torulae in the tissues, they differed in no important respect morphologically from those studied in culture. However, in the peritoneum encapsulated forms were extremely numerous, while in other organs they were rare.

Discussion

The literature pictures torulosis as a disease that is most often systemic with a cerebral localization. The organism is disseminated by the blood stream from a focus that is commonly found in the respiratory tract. In this form the disease is well known.

On the contrary the non-nervous forms are rare and are involved in certain obscurities. This is the reverse of the situation that exists in regard to the other fungus diseases in which the local manifestations are more common and have been more completely studied than the general manifestations. One may suspect that the picture of torulosis has been distorted because of the relative facility with which the cerebral form may be recognized in the course of a routine lumbar puncture and the difficulties that attend isolation of the organism from visceral or surface lesions.

The case here reported may be considered typical as regards the distribution and histology of the lesions and the bacteriological characteristics of the organisms. Moreover, it has been demonstrated that this typical Torula histolytica constantly produces characteristic lesions in mice. It is urged for this reason that in all Torula studies mice be used as the standard test animal. Only in this way can the results of different workers be made comparative.

The histology of the lesions of torulosis being quite unique, the mechanism by which the Torula acts on the tissues has intrigued certain authors. Stoddard and Cutler regarded the action to be chiefly lytic. Freeman, on the contrary, believed that the clear spaces in the tissues represented an accumulation of the mucoid material elaborated by the parasite. Our own studies leave us in some doubt, but on morphological grounds it appears that both mechanisms are in operation. The manner in which cystic lesions cut across gray and white matter (mice) and the occurrence of similar lesions in the pancreas (human case) where fluid could hardly collect under pressure, speak for lysis. But in the brain lesions there is clear evidence of pressure and even of infiltration about the lesions by fluid. Furthermore, after microincineration the products of liquefaction necrosis might be expected to leave a mineral residue which they do not do.

The degree and character of the inflammatory response that the Torula excites seem to vary greatly in different human cases. It may be feeble or absent, proliferative or occasionally exudative, and its character cannot be entirely explained by the age of the lesion. In our experimental animals the inflammatory response seemed to be almost entirely histiocytic and its degree related to the local supply of cells capable of being mobilized as macro-

phages. Few in the brain, such cells were more numerous in the lung and peritoneum. The same general statement, applied to our human material, would be an exaggeration, although not an excessive one.

The association of Hodgkin's disease and torulosis raises again the question of the role of infection in the etiology of the former. Because Hodgkin's-like changes have been produced experimentally with a diphtheroid bacillus by Bunting and Yates [50, 51] and with avian and bovine tubercle bacilli by L'Esperance [52] and Medlar,[53] we made a careful search for evidence of mobilization of megakaryocytes, but without success. Fitchett and Weidman [16] report a similar experience.

Concerning the organism itself, a few words at this point might not be out of place. A survey of the literature reveals that there is no specific agreement in regard to the fermentative capacity of the various strains. Pigmentation is also variable. Because of this, fermentation and pigment production can only be given general consideration in any scheme of classification. These variations may be evidence of dissociation. Sheppe [23] has called attention to the production of black "caps" on his colonies. In our own cultures we have observed active dissociation by way of secondary colonies. Fitchett and Weidman [16] mention the formation of mucoid colonies. Punkari and Henrici [54] have studied pigment changes in Torula. The detailed variations recorded by different investigators may therefore be related to separate phases of a cyclogeny, a fact well known to occur among the bacteria. In view of this fact, this question interposes itself: How may we recognize Torula histolytica for diagnostic purposes? We suggest that the following points are basic to this end: (1) reproduction by budding; (2) absence of mycelium; (3) absence of ascospores; (4) limited powers of fermentation without gas formation; and (5) demonstration of specific lesions in the mouse. In regard to point 3 it is realized that should the work of Todd and Herrmann [49] receive confirmation this criterion will vanish.

Experimental inoculation in mice rapidly results in lesions that are histologically highly specific and remarkably uniform. Because of this fact we suggest that intraperitoneal inoculation of these animals with spinal fluid or cultures should prove a useful method of quickly establishing an exact etiological diagnosis.

SUMMARY AND CONCLUSIONS

A review of the literature on torulosis reveals that the purely visceral and local forms of the disease, as opposed to the meningo-encephalitic types, have been incompletely studied.

The frequency and the course of these forms can be ascertained only by more complete laboratory studies along the lines suggested in this paper.

Our experience with a case of generalized torulosis indicates the desirability of employing mice routinely for inoculations. This is based on the great susceptibility of these animals and on the uniform and characteristic picture evoked. From this fact we suggest that intraperitoneal inoculation of mice should prove of value in establishing the etiological diagnosis.

REFERENCES

1. Stoddard, James L., and Cutler, Elliott C. Torula infection in man. A group of cases, characterized by chronic lesions of the central nervous system, with clinical symptoms suggestive of cerebral tumor, produced by an organism belonging to the Torula group (Torula histolytica, N. Sp.). *Studies Rockefeller Inst. M. Research, Reprints,* 1916, **25**, 1–98.

2. Hansmann, G. H. Torula infection in man. Report of a case. *Boston M. & S. J.*, 1924, **190**, 917–919.

3. Pierson, Philip H. Torula in man; report of a case with necropsy findings. *J. A. M. A.*, 1917, **69**, 2179–2181.

4. Gill, William D. Torula mycosis in man with special reference to involvement of the upper respiratory tract (with case reports). *Tr. Am. Laryng., Rhin. & Otol. Soc.*, 1934, **40**, 247–262.

5. Jones, E. Lloyd. Torula infection of the naso-pharynx. *South. M. J.*, 1927, **20**, 120–126.

   Jones, E. Lloyd. Torula infection of the palate and naso-pharynx. *West Virginia M. J.*, 1927, **23**, 184–187.

6. McGehee, J. L., and Michelson, I. D. Torula infection in man, report of a case. *Surg., Gynec., & Obst.*, 1926, **42**, 803–808.

7. Hall, George W., Hirsch, Edwin F., and Mock, Harry. Torula histolytica meningo-encephalitis. *Arch. Neurol. & Psychiat.*, 1928, **19**, 689–694.

8. Freeman, Walter, and Weidman, Fred D. Cystic blastomycosis of the cerebral gray matter caused by Torula histolytica Stoddard and Cutler. *Arch. Neurol. & Psychiat.*, 1923, **9**, 589–603.

9. Freeman, Walter. Torula infection of the central nervous system. *J. f. Psychol. u. Neurol.*, 1931, **43**, 236–345.

10. Swift, Harry, and Bull, Lionel B. Notes on a case of systemic blastomycosis — blastomycotic cerebro-spinal meningitis. *M. J. Australia*, 1917, **2**, 265–267.

11. Barlow, D. L. Primary blasto-mycotic meningitis occurring in a child. *M. J. Australia*, 1923, **2**, 302–304.

12. Bettin, Mona E. Report of a case of Torula infection. *California & West. Med.*, 1924, **22**, 98–101.

13. Watts, James W. Torula infection; a review and report of two cases. *Am. J. Path.*, 1932, **8**, 167–192.

14. Stone, Willard J., and Sturdivant, B. F. Meningo-encephalitis due to Torula histolytica. *Arch. Int. Med.*, 1929, **44**, 560–575.

15. Massee, Joseph C., and Rooney, J. Stewart. Meningitis due to Torula histolytica; report of a case. *J. A. M. A.*, 1930, **94**, 1650–1653.

16. Fitchett, Marion S., and Weidman, Fred D. Generalized torulosis associated with Hodgkin's disease. *Arch. Path.*, 1934, **18**, 225–244.

17. Cabot, Richard C. Case records of the Massachusetts General Hospital. 1934. *New England J. Med.*, 1934, **210**, 1291–1294.

18. Rusk, Glanville Y., and Farnell, Frederick J. Systemic oidiomycosis; a study of two cases developing terminal oidiomycetic meningitis. *Univ. California Publ. Path.*, 1912, **2**, 47–58.

19. Rappaport, B. Z., and Kaplan, Bertha. Generalized Torula mycosis. *Arch. Path. & Lab. Med.*, 1926, **1**, 720–741.

20. Williams, J. R. A case of systemic blastomycosis. *M. J. Australia*, 1922, **2**, 185–187.

21. Hirsch, Edwin F., and Coleman, George H. Acute miliary torulosis of the lungs. *J. A. M. A.*, 1929, **92**, 437–438.

22. Berghausen. Oscar. Torula infection in man. *Ann. Int. Med.*, 1928, **1**, 235–240.

23. Sheppe, W. M. Torula infection in man. *Am. J. M. Sc.*, 1924, **167**, 91–108.

24. Smith, F. B., and Crawford, J. S. Fatal granulomatosis of the central nervous system due to a yeast (Torula). *J. Path. & Bact.*, 1930, **33**, 291–296.

25. Rogers, James B., and Jelsma, Franklin. Torula meningo-encephalitis. *J. A. M. A.*, 1933, **100**, 1030–1031.

26. Wile, Udo J. Cutaneous torulosis. *Arch. Dermat. & Syph.*, 1935, **31**, 58–66.

27. White, E. C. A case of meningo-encephalitis due to Torula. *U. S. Nav. M. Bull.*, 1930, **28**, 615–618.

28. Semerak, C. B. Torula leptomeningitis. *Arch. Path.*, 1928, **6**, 1142–1145.

29. McKendree, Charles A., and Cornwall, Leon H. Meningo-encephalitis due to Torula. *Arch. Neurol. & Psychiat.*, 1926, **16**, 167–181.

30. Weidman, Fred D., and Ratcliffe, Herbert L. Extensive generalized torulosis in a Chetah or hunting leopard (*Cynaelurus jubatus*). *Arch. Path.*, 1934, **18**, 362–369.

31. Miller, J. E. Yeast-cell infection in man: report of case. *U. S. Nav. M. Bull.*, 1925, **23**, 229–235.

32. Goodheart, Philip S., and Davison, Charles. Torula infection of the central nervous system. *Arch. Neurol. & Psychiat.*, 1937, **37**, 435–439.

33. Johns, F. M., and Attaway, C. L. Torula meningitis; report of a case and summary of literature. *Am. J. Clin. Path.*, 1933, **3**, 459–465.

34. Weidman, Fred D. Cutaneous torulosis. *South. M. J.*, 1933, **26**, 851–863.

35. Lynch, Frank B., Jr., and Rose, Edward. Torula meningitis; report of an additional case. *Ann. Clin. Med.*, 1926, **4**, 755–760.

36. Evans, Newton. Torula infection. *California State J. Med.*, 1922, **20**, 383–385.

37. Brewer, George Emerson, and Wood, Francis Carter. Blastomycosis of the spine. Double lesion: two operations: recovery. *Ann. Surg.*, 1908, **48**, 889–896.

38. Shapiro, Louis L., and Neal, Josephine B. Torula meningitis. *Arch. Neurol. & Psychiat.*, 1925, **13**, 174–190.

39. Flu, P. C., and Woensdregt, M. M. G. Een Geval van Blastomycose van het Centraalzenuwstelsel. *Mededeel. v. d. Burgerl. Geneesk. Dienst in Nederl.-Indië*, 1918, Deel **6**, 1–26.

40. Wortis, S. Bernard, and Wightman, Henry B. A case report of Torula meningitis. *Bull. New York Acad. Med.*, 1928, **4**, 531–536.

41. Mitchell, Louis A. Torulosis. *J. A. M. A.*, 1936, **106**, 450–452.

42. Urbach, E., and Zack, F. Generalisierte Torulose (europaïsche Blastomykose). Eine klinisch-botanische Studie. *Arch. f. Dermat. u. Syph.*, 1930, **162**, 401–421.

43. Alvarez, Ruth Stone. A red Torula as the cause of a tongue abnormality. *J. A. M. A.*, 1926, **87**, 1358–1359.

44. Tabor, Kenneth W. Torulosis in man; report of a case. *J. A. M. A.*, 1937, **108**, 1405–1406.

45. Badham, Charles. Preliminary report on the causal organism of blastomycotic meningitis in Australia. *M. J. Australia*, 1922, **2**, 385.

46. Ball, Howard A. Human Torula infections: a review; report of cases. *California & West. Med.*, 1930, **32**, 338–346.

47. Langeron, Maurice. Mycétome a Torula Jeanselmei Langeron, 1928. Noveau type de mycétome a grains noirs. *Ann. de parasitol.*, 1928, **6**, 385–403.

48. Dodge, Carroll William. Medical Mycology. C. V. Mosby Company, St. Louis, 1935.

49. Todd, Ramona L., and Herrmann, Walter W. The life cycle of the organism causing yeast meningitis. *J. Bact.*, 1936, **32**, 89–103.

50. Bunting, C. H., and Yates, J. L. An etiologic study of Hodgkin's disease; preliminary note. *J. A. M. A.*, 1913, **61**, 1803–1804.

51. Bunting, C. H., and Yates, J. L. An etiologic study of Hodgkin's disease; second note. *J. A. M. A.*, 1914, **62**, 516–517.

52. L'Esperance, Elise S. Studies in Hodgkin's disease. *Ann. Surg.*, 1931, **93**, 162–168.

53. Medlar, E. M. An interpretation of the nature of Hodgkin's disease. *Am. J. Path.*, 1931, **7**, 499–514.

54. Punkari, Laila, and Henrici, Arthur T. A study of variation in a chromogenic asporogenous yeast. *J. Bact.*, 1933, **26**, 125–138.

DESCRIPTION OF PLATES

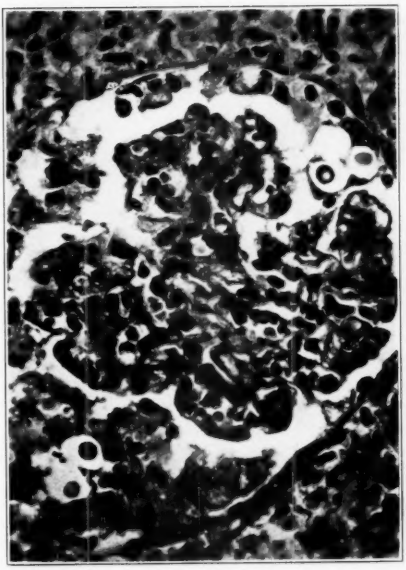
PLATE 122

FIG. 1. Lateral surface of the left frontal lobe. Thickening and clouding of the meninges, most marked along the cerebral vessels.

FIG. 2. Multiple small cysts lateral to the ventricles.

FIG. 3. Miliary lesions in the kidney.

FIG. 4. Torulae in a glomerulus. The large forms are surrounded by endothelial leukocytes; the small forms are intracellular. × 450.



Crone, DeGroat and Wahlin Torula Infection

UNIV. OF MICH.

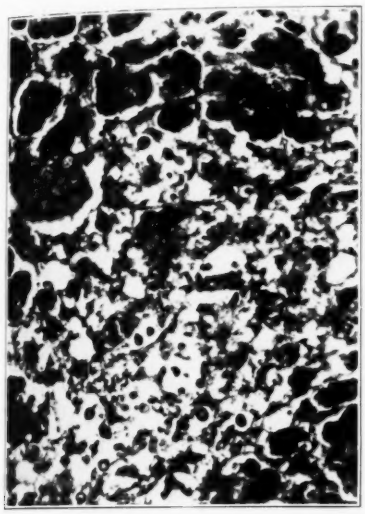
PLATE 123

FIG. 5. Dilated renal tubule containing Torulae. × 450.

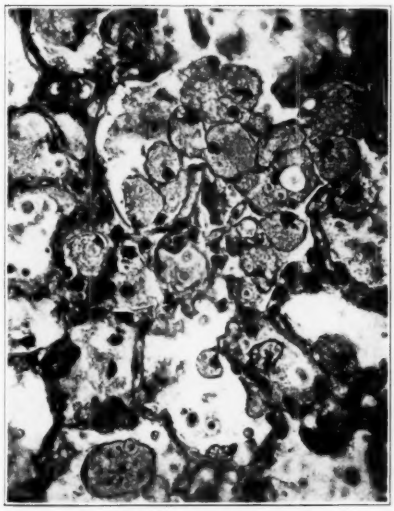
FIG. 6. Lesion in the pancreas showing the absence of inflammatory reaction. × 450.

FIG. 7. Cystic lesion in the brain of a mouse. The cyst is traversed by a capillary containing a Torula. × 450.

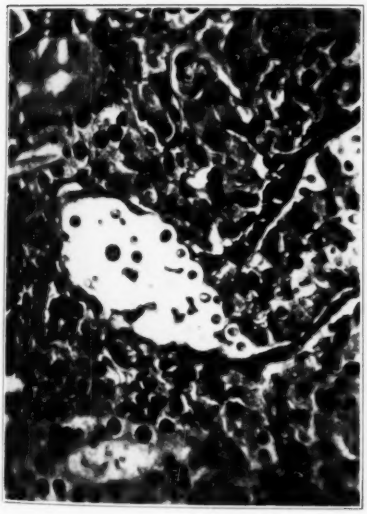
FIG. 8. Torulous pneumonia in the mouse. There are numerous minute intracellular organisms. The exudate consists of macrophages having a foamy cytoplasm. × 450.



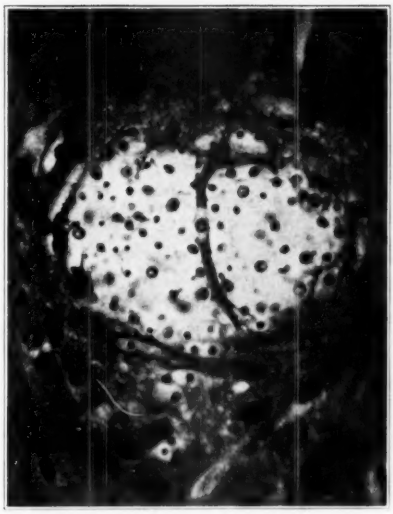
6



8



5



7

Crone, DeGroat and Wahlin Torula Infection

UNIV. OF MICH.